BULLETIN OF THE TORREY BOTANICAL CLUB
Vol. 85, No. 3, pp. 157–178 May, 1958

A COMPARATIVE CYTOLOGICAL STUDY OF FOUR SPECIES OF CHLAMYDOMONAS[1]

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Relatively few investigators have engaged in cytological studies of *Chlamydomonas* and karyological investigations in particular have been neglected. Due to the extremely small size of the chromosomes, and because conventional techniques for staining and identifying individual chromosomes have not always yielded good results, those reports which have been made of chromosome counts in various species are confusing and contradictory.

Dangeard (1899) described in detail the morphology of several volvocacean species, giving particular attention to the mitotic process. He reported the following (haploid) chromosome numbers for species of *Chlamydomonas:*

Chlamydomonas monadina	30.
Chlamydomonas variabilis	10.
Chlamydomonas dilli	10.

Among other observations, one which appears to be important in connection with the present work was that the formation of chromosomes in these species is preceded by a stage at which the number of chromatinic granules is larger than that of the chromosomes as seen at their fully formed, condensed condition.

In a lengthy study of the comparative morphology of several protozoa, algae and fungi, Bêlaŕ (1926) gave passing attention to *Chlamydomonas.* In one of his figures of an unidentified species, 8 anaphase chromosomes are depicted passing toward each pole.

Kater (1929), in a study of the cytology of *Chlamydomonas nasuta,* reported the chromosome number to be 8. Like Dangeard, he found that the appearance of a large number of chromatinic granules during early prophase is characteristic of mitosis, and that condensation of these leads to the development of fully formed, late prophase chromosomes. He reported the appearance of centrioles and spindle fibers. According to his account, the nuclear membrane does not disappear until late anaphase, a

[1] This paper is a portion of a Ph.D. thesis submitted to the Graduate School of Vanderbilt University, Nashville, Tennessee. The work was supported in part by a grant from the Southern Fellowships Fund.

[2] The author wishes to express appreciation to Dr. H. C. Bold for his assistance during the course of investigation and for help in preparing the manuscript. He is also indebted to Dr. R. J. Neff for his valuable suggestions.

[The Bulletin for March–April 1958 (85: 84–156) was issued May 21, 1958.]

phenomenon which is not in general agreement with the observations of other investigators. Kater also found that at the second division both cells divide at right angles to the original plane of cleavage and at right angles to each other.

In reviewing the work of Franz Moewus on the nature of sexuality in *Chlamydomonas,* Hartmann (1934) reported the chromosome numbers of *C. paupera* and *C. eugametos,* which Moewus succeeded in crossing, as 10 in each species; according to this account, the hybrid of this cross also exhibited this number, and behavior of a sex chromosome was described. Moewus (1936), himself, reviewed this same work and included data concerning the inheritance of certain morphological, physiological and zygotic characters, reporting specific cases of genetic linkage. The figures accompanying this paper include a zygote in the first meiotic division exhibiting 10 bivalents. In a later publication (1940), Moewus presented evidence for the existence of 10 linkage groups in *C. eugametos* obtained by crossing various mutants.

Akins (1941) made a cytological study of *Carteria crucifera* which, along with other members of this genus, is morphologically similar to *Chamydomonas,* the most obvious difference being that the former is quadriflagellate and the latter biflagellate. One of the goals of Akins' study was to determine whether any correlation could be found between the double number of flagella in *Carteria* and the chromosome number as compared with that of *Chlamydomonas* species. Inasmuch as Akins found the chromosome number in this species to be $n = 9$, and since previous reports of chromosome counts in *Chlamydomonas* ranged from 8 to 30, no relation between chromosome number and the number of flagella was obvious. However, the chromosome number itself is of interest, as are many other details which are reported. Nuclear division was described by Akins as occurring after a revolution of 90° by the protoplast, just before which the flagella had been either lost or withdrawn. The single pyrenoid usually divided before the nucleus. Prophases of division were characterized by the appearance of numerous chromatinic granules. The nuclear membrane and nucleolus disappeared at this stage, and definitely organized chromosomes then arranged themselves on the equatorial plate. At telophase, nucleoli reappeared and nuclear membranes were formed about each group of chromosomes. Although a number of different stains were employed in Akins' study, centrioles were not observed. In second division, the two nuclei did or did not divide simultaneously, according to this investigator.

Cave and Pocock (1951a), in studying nuclear behavior in the colonial Volvocales, have reviewed accounts in which chromosome numbers are given for members of this group previous to their own investigations. They succeeded in counting the chromosomes of several species, the cells of which

bear some resemblance to those of *Chlamydomonas*. Chromosome numbers in this group are consistently low, ranging from 5 in several species of *Volvox* to 17 in *Gonium pectorale*. No essential variation in the mitotic cycle was observed among these species, and it may be summarized as follows. At the beginning of prophase, small chromatinic bodies appeared throughout the nucleus. The nucleolus was well stained, but became fainter as prophase progressed. The attenuated chromosomes, of which the chromatinic bodies are a part, shortened and thickened, reaching maximum contraction at metaphase, where they became aligned on the equator. Centrioles were not observed. The aceto-carmine technique, which Cave and Pocock used exclusively, failed to reveal the presence of spindle fibers. The nuclear membrane disappeared before metaphase and reappeared with the formation of daughter nuclei. Telophase chromosomes stained heavily, but rapidly lost their stainability as the nuclei proceeded toward interphase. After the nucleus had divided, nucleoli reappeared in the daughter nuclei, and cytokinesis followed. Cave and Pocock concluded that mitosis in these species is similar to that in higher plants.

Schaechter and DeLamater (1954, 1955) studied mitosis in 4 species of *Chlamydomonas*, reporting chromosome numbers as follows:

C. moewusii Gerloff	36 ± 2.
C. eugametos Moewus	36 ± 4.
C. reinhardti Dangeard[3]	18 ± 2.
C. dysosmos Moewus	16 ± 1.

They found the pattern of mitosis to be similar in all 4 species and essentially like that in most plants. Details of the process are described briefly; nothing is said of the fate of the nuclear membrane, nucleolus or spindle fibers, nor is the presence of centrioles reported.

These investigators also studied meiosis in *C. moewusii* (1954, 1956). They described configurations which are suggestive of diplotene loops, the number of which appeared to be on the order of 8 to 10. In view of the difficulty of reconciling this number with their previous haploid chromosome count of 36 ± 2, these authors suggest that meiosis in this organism cannot be explained according to a classic pattern, or if it does occur as such, that the difficulty of resolving such small chromosomes precludes a correct interpretation.

In a study of polyploidy in *C. eugametos* and *C. reinhardti*, Wetherell and Krauss (1956) reported the chromosome number to be 16 in *C. reinhardti*.

Materials and methods. Stock cultures of three of the four organisms used in this study, *C. eugametos* Moewus, *C. reinhardti* Dangeard and *C.*

[3] Trainor (1957) states that the correct specific epithet is *"reinhardti,"* not *"reinhardi"* as usually given.

moewusii Gerloff, were maintained in bacteria-free cultures on slants of Knop's agar, compounded as follows:

10% $Ca(NO_3)_2$	10 ml.
5% KNO_3	5 ml.
5% $MgSO_4 \cdot 7H_2O$	5 ml.
5% KH_2PO_4	5 ml.
Distilled water	1575 ml.
Agar-agar	24 gm.

C. chlamydogama Bold, which has been shown to be deficient for vitamin B_{12} (Hutner *et al* in Lwoff 1951, Bell 1953), was grown successfully on Knop's agar supplemented with soil extract. In some cultures of *C. chlamydogama* vitamin B_{12} was supplied as "Cobione" (Merck and Company) at the rate of 15 micrograms/150 ml. of Knop's agar.

Cultures were grown at temperatures of 19° to 22° C. Except where otherwise specified, illumination was at an intensity of 800 foot-candles produced by a battery of "daylight" fluorescent bulbs which burned continuously. Stock cultures were maintained, however, at 100 foot-candles for 12 hours daily.

In preparing cultures of organisms for staining, the following method was employed. Cultures were grown for three days on appropriate media, at the end of which a quantity of material was transferred and spread over the same medium in fresh culture dishes. The maximum number of division figures was obtained in all species at 8–16 hours' illumination after such transfer.

Glass slides were cleaned in a solution of acid alcohol, wiped dry, and one end of each slide was smeared with egg albumin. After drying, a few drops of one of the fixatives described below were placed upon the end of the slide, into which cells were introduced, having been removed from the culture plate by a platinum loop or edge of a cover glass. When the cells were just at the point of drying, the slide was immersed in a Coplin jar of the same fixative into which the cells had first been introduced.

Because the small size of the chromosomes of these species necessitates extreme care in preparation and observations, four different techniques were employed in the preparation of material of nuclear and cell division. These were the iron-alum haematoxylin, Feulgen, Azure A (DeLamater 1951) and aceto-carmine techniques. Two modifications of the last named method were developed for use in the present work. The first, which is a modification of the technique developed by Cave and Pocock (1956) as applied to the colonial Volvocales, was used whenever cellular details were to be studied. The fixative, which Cave and Pocock modified from Johansen (1940), is prepared as follows:

Iodine	0.5 gm.
Potassium iodide	1 gm.
Acetic acid, glacial	4 ml.
Formalin	24 ml.
Distilled water	400 ml.

Slides were allowed to remain in the fixative for at least 15 minutes to about 5 days; after longer periods in the fixative the cells did not respond satisfactorily to further treatment. Upon removal, excess fluid was drained from the slide, two drops of aceto-carmine prepared according to the method described by Cave and Pocock (1951b) were placed upon the area occupied by fixed cells, a cover glass was gently lowered, and the slide heated over a low flame. It was found that the method of heating followed by Cave and Pocock (1951b), namely, allowing the fluid to reach almost the boiling point, did not result in stained chromosomes in any of the species of *Chlamydomonas* which were utilized. Staining was inadequate unless the fluid beneath the cover glass was actually boiled. In utilizing this method, great care must be taken to prevent overstaining; the exact and delicate point to be reached between under-staining and over-staining was learned in the present study only after much trial and error.

The second aceto-carmine method which was developed for use in the present work is an adaptation of the technique developed by Ray (1956) in staining *Tetrahymena* cells. While this method brought out few cellular details, it was superior in the staining of chromosomes to the other aceto-carmine method described above. Thus the two methods supplemented each other admirably in the present work.

Since considerable modification of Ray's method was required, it is felt that a detailed account of it also should be given. In fixing cells, a drop of distilled water was transferred to a glass slide, into which were deposited a quantity of cells which had been scraped from the surface of a plate by means of a clean cover glass. These were distributed as evenly as possible within the drop of water. Next, a small drop of Nissenbaum's Fluid (Nissenbaum 1953) was placed upon the suspension of cells and thoroughly mixed with a dissecting needle. As soon as evaporation almost to the point of dryness had occurred, the slide was immersed in a Coplin jar of Carnoy's 1:3 acetic acid-absolute ethyl alcohol. Primary fixation in Carnoy's solution did not prove to be quite as satisfactory as did the use of Nissenbaum's Fluid.

Glass slides containing cells fixed by this method were stored in Carnoy's solution for 1–24 hours, after which time they were transferred to 70% ethyl alcohol, where they could be left indefinitely. Upon carrying the slides to completion, they were transferred to 50%, 30% and 15% ethyl alcohols,

and then into water. Hydrolysis then was accomplished by placing the slides in 1-normal hydrochloric acid in the following series: 2 minutes at room temperature, 6 minutes at 60° C., and 2 minutes a second time at room temperature. Following this treatment, the slides were washed in at least 5 changes of distilled water within a period of 5 minutes.

Slides then were transferred to 45% acetic acid, where they were allowed to remain for 5–7 minutes. Following this treatment, they were removed to a Coplin jar containing aceto-carmine stain, where they were stored for

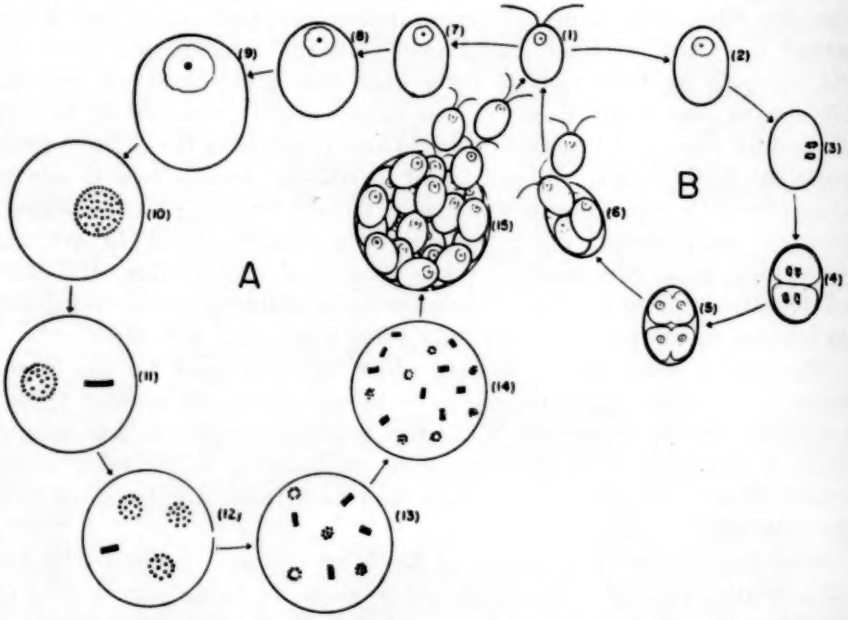


FIG. 1. Diagram to illustrate the effects of light intensities of 800 as compared with 100 foot-candles on *Chlamydomonas eugametos* and *C. moewusii*. The cycle at A (1, 7–15) occurs at 800 foot-candles and that at B (1–6) at 100 foot-candles or less. The cycle at B reaches stage (3) approximately 4 hours after the cultures are inoculated. Note relatively small size of cell at inception of division (3) as compared with a similar stage (10) in cycle A. In the cycle at B, 8 chromosomes are visible at each mitosis. In cycle A, nuclear and cell division are delayed through a period of 8–12 hours during which cell enlargement and endomitosis (presumably) occur. Note the progressive decrease in chromosome number at successive mitoses between (10) and (14) and formation of 32 cells (15) as compared with 4 or 8 at 100 foot-candles (6). See text for amplification.

at least 4 hours to obtain adequate staining; 4–8 hours in the stain proved to be ideal. If slides were allowed to remain in the stain for a longer time than this, the cells tended to become overstained. However, good results could be obtained from slides which had been in the stain for several days if, before being heated, they were removed to 45% acetic acid for a period which had to be determined experimentally, a period proportional to the

time the slide had been in the stain. This varied in the present work from about 1 to 8 hours.

Following the 4–8 hour period of staining, or after the slides had been placed in 45% acetic acid for an appropriate length of time as described above, they were removed individually for heating. Excess stain was wiped from the slide, an additional drop of aceto-carmine was placed on the area occupied by the cells, and a cover glass gently lowered. Heating did not need to be as intense as in the modified Cave and Pocock method described above, but gentle boiling for an instant usually was necessry.

In employing both of these methods, it was found that best results were obtained from a study of cells which had been freshly stained, and all drawings and photographs which were made in connection with the present study were made from such material. Preparations could be rendered permanent with a certain degree of success, however, by following the method of Manton (1950).

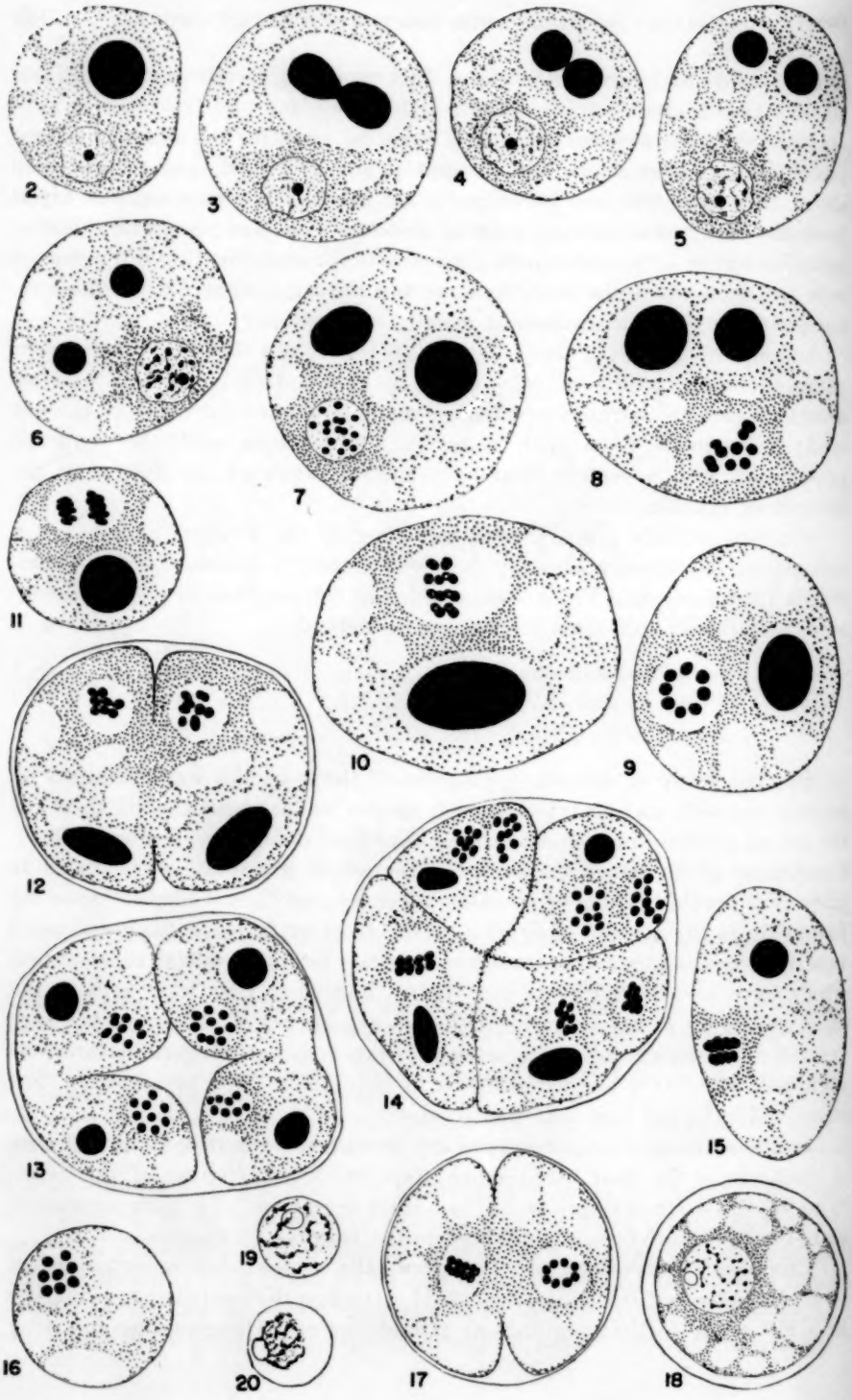
Fixation of cells preparatory to staining by the Feulgen and Azure A techniques was accomplished by means of Carnoy's solution, while Schaudinn's Protozoological Fluid, compounded as follows, was employed in connection with the iron-alum haematoxylin method:

Saturated aqueous $HgCl_2$	200 ml.
Ethyl alcohol, 95%	100 ml.
Acetic acid, glacial	9 ml.

For the study of meiosis, zygospores of the 4 species were obtained by mixing opposite mating types of each species under optimal conditions for the sexual reaction in each case which have been defined by Trainor (1957). Conditions under which these zygospores would germinate were found to vary considerably. Those of *C. chlamydogama* and *C. reinhardti* responded favorably to the method of Starr (1949); those of *C. moewusii* germinated readily when they were produced within the limits of initial illumination which have been stated by Lewin (1949), and when they were kept in the dark according to his methods. Although zygospores of *C. eugametos* were treated in a variety of ways, a very low percentage of germination was obtained. The Starr method resulted in only about 10% germination, but it proved to be the best that was tried.

In the staining of zygotes, only the iron-alum haematoxylin and Azure A methods, of the four listed above, were employed. Neither of the variations of the aceto-carmine technique, used so successfully with vegetative cells, could be satisfactorily adapted to the staining of zygotes.

Comparative cytology of vegetative cells. Cytokinesis is accomplished in all 4 species by furrowing (figs. 12, 17), the first division always occurring at a 90° angle to the longitudinal axis of the cell. Observations on living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cells of the 4 species reveals that the protoplast turns 90° within the cell wall just prior to the first nuclear division, a phenomenon also described by Kater (1929) for *C. nasuta* and by Akins (1941) for *Carteria crucifera*. The second division generally occurs at right angles to the first (fig. 13). It seems evident that cytokinesis begins most readily in areas where the cytoplasm is least dense, and since this often is true in the central portion of the cell, the process may begin there and at the periphery of the cell simultaneously.

There is no exact synchrony between division of the nucleus and division of the pyrenoid. Within a given population, some cells may be seen whose pyrenoids are dividing but whose nuclei are not (figs. 3, 35) or the reverse may be true (fig. 11). In general, it was observed that in cells which are grown at a light intensity of 100 foot-candles, division of the pyrenoid precedes nuclear division (figs. 2–8), while in cells which are subjected to a light intensity of 800 foot-candles nuclear division precedes division of the pyrenoid (figs. 9–11). In the formation of daughter cells, nuclear and pyrenoidal divisions eventually become adjusted so that before each cytokinesis, nuclei and pyrenoids always lie near each other in pairs (fig. 13).

Observation of the behavior of the stigma during division of living cells revealed that one daughter cell retains the original stigma, and the other division products develop theirs *de novo*.

Cytokinesis usually is initiated between the two contractile vacuoles, one of the latter being transmitted to each new daughter cell. Although the process was not observed, it is assumed that each cell develops an additional contractile vacuole, as two are apparent very shortly after division. The details regarding behavior of the pyrenoid, stigma and contractile vacuoles during cell division are essentially similar in the 4 species studied.

Preliminary observations indicated that *C. reinhardti* was most favorable for the study of mitosis, chiefly because greater numbers of division

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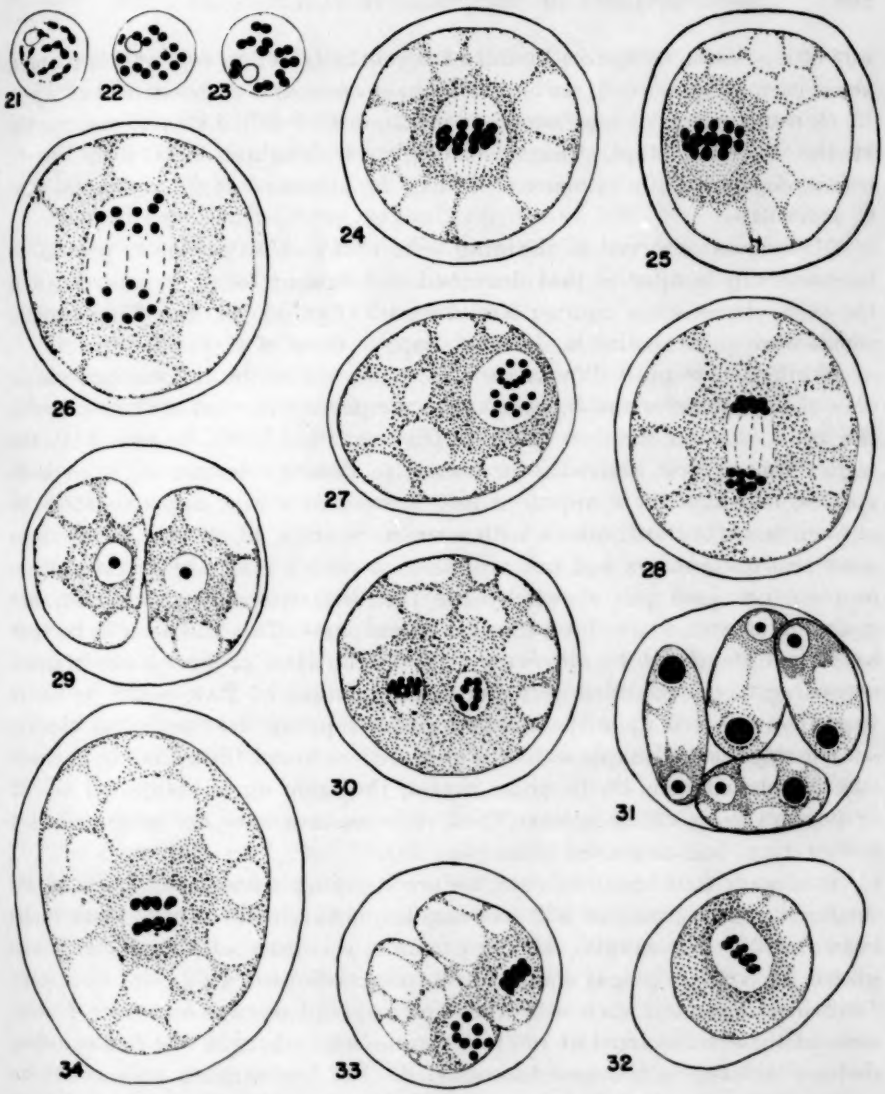
FIGS. 2–14. *Chlamydomonas reinhardti*. FIGS. 15, 18–20. *Chlamydomonas chlamydogama*. FIG. 16. *Chlamydomonas eugametos*. FIG. 17. *Chlamydomonas moewusii*. FIGS. 2–17. Mitosis. FIGS. 2–8. Nuclei in successive stages of prophase showing condensation of chromatin into chromosomes, division of pyrenoid. FIG. 9. Nucleus in metaphase, 8 chromosomes in ring formation (polar aspect); pyrenoid undivided. FIGS. 10, 11. Anaphasic separation of two rings of 8 chromosomes. FIG. 12. Late prophase of second division. FIG. 13. Late prophase of third division. FIG. 14. Late prophase of fourth division exhibited by 6 nuclei, one nucleus still in early anaphase of the previous division. FIG. 15. Anaphasic separation of two rings of 8 chromosomes. FIG. 16. Late prophase, one chromosome yet unoriented in ring. FIG. 17. Metaphase of second division, 8 chromosomes in ring formation in each nucleus. FIGS. 18–20. Meiosis; nuclei in successive stages of prophase showing condensation of chromatin. FIG. 20. Chromatin condensed into synizetic knot (compare Figs. 55, 56). FIGS. 2–17. drawn with aid of camera lucida from aceto-carmine preparations; FIGS. 18–20. drawn from iron-alum haematoxylin preparations. All figures ×3035.

figures could be obtained in cells of this species than in others. This may be an indication that the process of mitosis requires more time in cells of *C. reinhardti* than in cells of *C. chlamydogama*, *C. eugametos* and *C. moewusii*. *C. reinhardti* proved to be a fortunate choice, not only for this reason, but also because there is no complicating phenomenon evoked at high light intensity such as appeared later in connection with cells of *C. eugametos* and *C. moewusii*, a feature which is to be described in detail later in this report.

The mitotic process is illustrated for *C. reinhardti* in figures 2–14 and figures 36–39. The interphase nucleus (fig. 2) is approximately 3 μ in diameter and exhibits one nucleolus. Azure A-positive granules are scattered about the nucleus or organized into short thread-like groups (fig. 2). During early prophase (figs. 3–5), these become organized into indefinite groups, which eventually condense in later prophase (fig. 6) into scattered bodies which are loosely connected by threads. Still later (fig. 7), a number of bodies, approximately twice the haploid chromosome number, are seen within the nucleus. Meanwhile, the nucleolus disappears (fig. 7) as does the nuclear membrane (fig. 8) and the condensing chromatinic bodies lie within a clear area, about which there is an accumulation of dense cytoplasm. At late prophase (fig. 8), it is common to observe 8–12 large bodies, but upon final condensation at metaphase, a ring of 8 chromosomes[4] is seen in polar aspect (figs. 9, 37, 38). It seems probable that a condensation process occurs between the prophase stage represented by figure 7 and metaphase as illustrated by figure 9, and that the variable number of "chromosomes" as seen at late prophase (fig. 8) is due to a lag in condensation on the part of some of these bodies. Figure 36 shows a cell of *C. reinhardti* with two nuclei in very late prophase of the second division, that to the left exhibiting a ring of 7 chromosomes with an eighth yet in the center, while the other nucleus, which is not in exact focus in this illustration, exhibits about 10 bodies. Figure 39 shows a cell whose nucleus is in anaphase of division, 4 chromosomes being seen in each separating group in this lateral view. There are, of course, 4 other chromosomes in each group which are not visible in this focus (fig. 10).

In cells which are grown at a light intensity of 800 foot-candles, cytokinesis usually lags somewhat behind mitosis; in figure 11, there is no evidence that cytokinesis has begun, even though the nucleus is in anaphase of division, and in figure 12, in which the two nuclei are in late prophase, cytoplasmic division following the first mitosis is just beginning. This same

[4] Although the writer is reasonably certain of the chromosome numbers, as stated in this paper, their extremely minute size makes it impossible to be absolutely sure. Therefore, when any number is stated, it should be considered the approximation which can be made under the conditions of observation. In any case, the error would be in the range of 1 or 2, at most.



FIGS. 21–31. *Chlamydomonas chlamydogama.* FIG. 32. *Chlamydomonas moewusii.* FIG. 33. *Chlamydomonas reinhardti.* FIG. 34. *Chlamydomonas eugametos.* FIGS. 21–34. Meiosis (continued). FIG. 21. Chromatin condensing into connected pairs of bodies. FIG. 22. Late prophase, paired chromatinic bodies no longer visibly connected. FIG. 23. Diakinesis, 8 bodies visible. FIG. 24. First metaphase, lateral aspect; chromosomes arranged in ring of 8 on spindle. Compare with figure 59. FIGS. 25, 26. Anaphasic separation of 2 rings of 8 chromosomes. FIG. 27. Anaphase, 2 rings of 8 chromosomes slightly flattened. Compare with figure 61. FIG. 28. Telophase. FIG. 29. End of first division, cytokinesis completed. FIGS. 30, 31. Second division. FIG. 32. Metaphase of first division showing ring of 8 double chromosomes. FIG. 33. Metaphase of second division, nucleus at upper right in lateral aspect showing ring of 8 chromosomes; nucleus at lower left in polar aspect, ring of 8 chromosomes slightly crushed and distorted. FIG. 34. Anaphase of first division, lateral aspect, showing 2 separating rings of 8 chromosomes. Compare with figure 62. Camera lucida drawings made from iron-alum haematoxylin preparations. All figures × 3035.

sort of lag is seen in figures 13 and 14. Eventually, a new cell wall is formed about each daughter cell, the old wall breaks open, and each new cell escapes. In *C. reinhardti*, this may occur at the 2-, 4- or 8-celled stages, and rarely at the 16-celled stage. Characteristically, 4 daughter cells form in *C. chlamydogama*, while as many as 32 may be produced in *C. eugametos* and *C. moewusii*.

Mitosis, as observed in dividing cells of *C. chlamydogama*, proved to be essentially similar to that described and figured for *C. reinhardti*, and the same chromosome number was observed (figs. 15, 40, 41). The chromosomes were quite similar in size and shape to those of *C. reinhardti*.

While no essential differences were observed in the mitotic process in cells of *C. eugametos* and *C. moewusii* as compared with that of *C. reinhardti*, the basic haploid number being 8 (figures 16, 17, 42, 44, 45, 51), the writer was at first somewhat perplexed to discover certain cells, in both species, in which there appeared metaphase plates with approximately 16 chromosomes and still others with even more (figs. 43, 46–50). That these were true metaphases and not prophases in which maximum condensation had not occurred was shown by the fact that the plates were flat, not rounded, as would have been the case in prophase. This was seen to be true by focusing and also by observing cells which, when prepared and stained according to the modified aceto-carmine technique of Ray, could be made to change position upon the slide by gentle tapping. In the lateral aspect, such metaphase or anaphase plates appeared as broad lines similar to those illustrated in figure 46. In polar aspect, the same nuclei exhibited 16, 32 or possibly more chromosomes. These chromosomes were not arranged in a hollow ring, but as a solid plate (fig. 50).

It already had been observed, before studying mitosis, that cells of all 4 species, when grown at 800 foot-candles, behaved differently from those grown at 100 foot-candles. If, for example, a culture of *C. moewusii* was grown upon a Knop agar slant at 100 foot-candles until all cells were uniform in size, and if such cells then were spread upon Knop agar plates, some of them maintained at 100 foot-candles and others at 800 foot-candles, distinct differences became observable. At 100 foot-candles, cells could be seen to undergo a period of growth of about 4 hours' duration, after which widespread division was in evidence. However, cells did not appear to reach a size exceeding twice their original volume before dividing. At 800 foot-candles, no division was evident at all before 10 hours' growth under the new conditions, and most cells did not divide before 15 hours. Some cells failed either to grow or divide. Figures 52–54 show cells of *C. moewusii* which enlarged in the manner indicated; the extent of growth of the large cells thus may readily be seen.

With some minor variations, this pattern was observed in all 4 species.

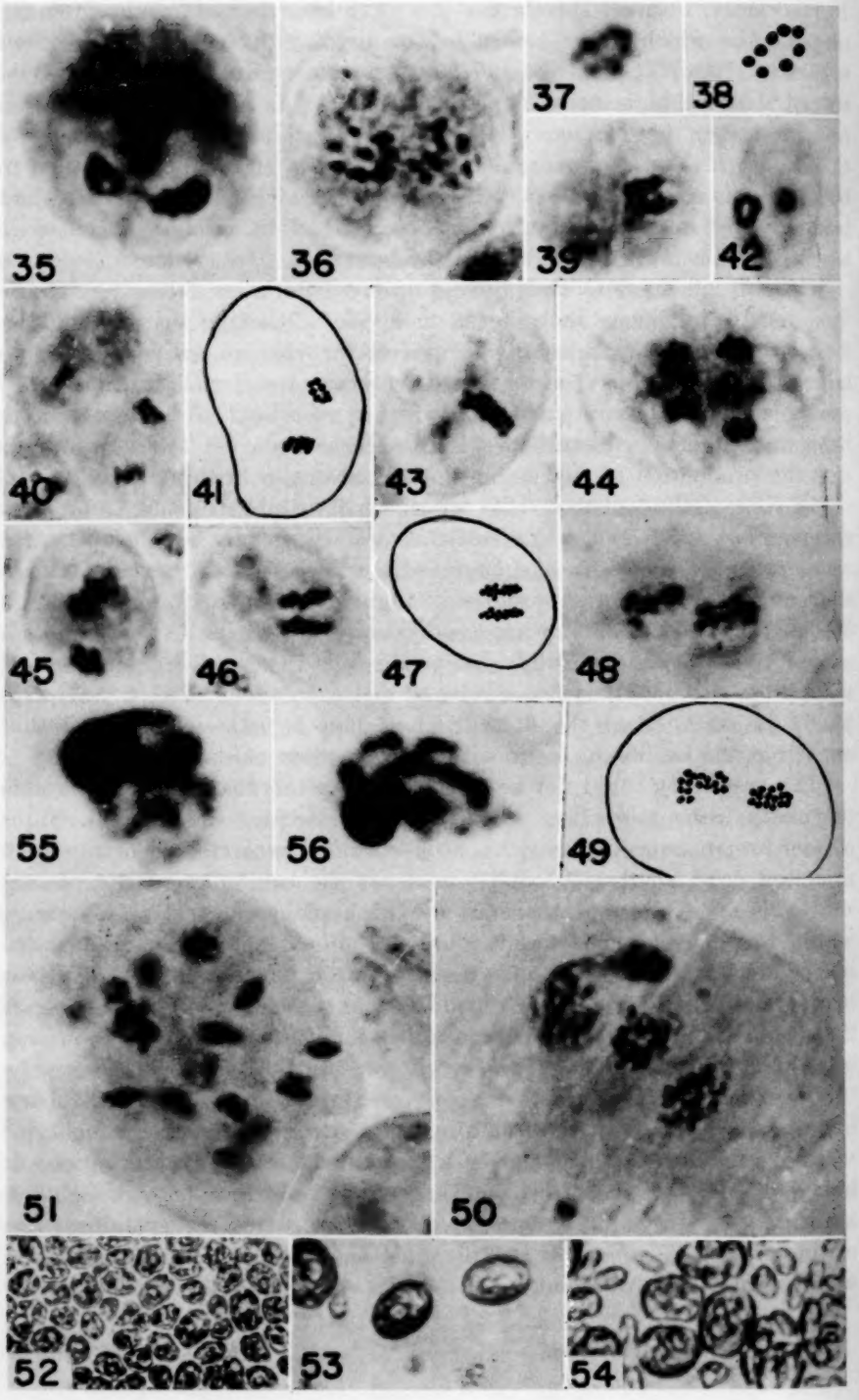
Furthermore, a direct correlation was established between light intensity and the size which cells attained befare dividing, by growing cells of all species at 100, 200, 400, 600 and 800 foot-candles. In general, it was observed that the higher the intensity, the longer cells remained undivided and the larger they became. Cells of *C. eugametos, C. chlamydogama* and *C. reinhardti* showed no more marked response to 800 foot-candles than to 600, whereas cells of *C. moewusii* did, becoming larger before division and taking longer to initiate the division process than did cells of other species as long as they were maintained in the light.

Cells of all species, when placed in total darkness following intense light treatment, immediately began to divide, indicating that light intensities which produce unusually large cells provide an artificial block to mitosis and cytokinesis; the continued synthesis of materials within the cell causes it to grow to an unusual size when in such light, division seemingly being inhibited by a disturbance of balance between the rate of synthesis and the production of division-initiating substances within the cell.

In view of the fact that those nuclei which exhibited about 16 or more chromosomes in *C. eugametos* and *C. moewusii* usually were observed in comparatively large cells, and because they were seen at the earliest divisions of these cells as they progressed toward the production of 8, 16 and 32 daughter cells, the following hypothesis suggested itself: a light intensity of 800 foot-candles, while inhibiting cell division for several hours, induces the cells of these two species to undergo endomitosis, so that polyploidy is exhibited at the first division. This hypothesis suggested that somatic reduction would occur with the formation of daughter cells.

The possibility could not be ruled out, however, that genetically stable polyploid strains had arisen in these species. Because the original strains of each organism maintained as stock cultures were clonal, having been produced from single-cell isolates made at the beginning of the present study, the possibility of the existence of haploid and polyploid strains within the stocks seemed highly unlikely, unless such strains had arisen independently within stock cultures after the original isolations. Furthermore, such an event would have had to occur 4 times independently, since the phenomenon of chromosomal multiplication was observed in both mating types of *C. eugametos* and *C. moewusii.*

Nevertheless, in order to rule out such a possibility altogether, new isolations were made in the following manner. Cells of each mating type, taken from cultures which had been maintained for many generations at low light intensity, and which had never been subjected to high light intensities, were spread on Knop agar plates. Two plates, one containing one mating type of *C. eugametos* and the other one mating type of *C. moewusii,* were grown at 800 foot-candles. Two plates containing the opposite mating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type in each case were grown at 100 foot-candles. After 18 hours' growth, 8 isolations were made from each dish, single cells being picked up and transferred to small tubes with sterile medium. In the case of those cells which had been grown in the brighter light, an attempt was made to isolate as large cells as possible, since it had been indicated that it was within such large cells that the higher chromosome numbers could be found.

Within 10 days, growth was evident in most of the tubes. One tube was selected from each of the 4 types of cells which were isolated, cells were concentrated by centrifugation, and spread upon Knop agar plates. These then were exposed to a light intensity of 800 foot-candles. Within 12 hours, a similar situation to that shown in figure 53 was evident in each of the 4 plates. This was considered presumptive evidence against the hypothesis that stable polyploid strains were involved from the beginning. Fixation and staining of these cells revealed in all four cases the presence of nuclei some of which exhibited the haploid number (8) and others of which were characterized by larger numbers at metaphase and early anaphases. On the basis of this experiment, therefore, the phenomenon of chromosomal multi-

FIGS. 35–39. *Chlamydomonas reinhardti.* FIGS. 40, 41, 55, 56. *Chlamydomonas chlamydogama.* FIGS. 42–45. *Chlamydomonas eugametos.* FIGS. 46–54. *Chlamydomonas moewusii.* FIG. 35. Division of pyrenoid. ×2650. FIG. 36. Two nuclei in prophase, that at left showing ca. 7 chromosomes in a ring; the 8th is yet unoriented. Right-hand nucleus not in exact focus; 10–12 fodies in late prophase. ×2650. FIG. 37. Nucleus in metaphase (polar aspect) showing ring of 8 chromosomes. ×5500. FIG. 38. Interpretive drawing of figure 37. ×5500. FIG. 39. Second anaphase (only 1 nucleus visible) in lateral view. Four chromatids visible in each separating ring of 8. ×2650. FIG. 40. Lateral aspect of two nuclei in late anaphase showing two separating rings of 8 chromosomes; note lateral position of division figure effected by earlier migration of nucleus. ×2650. FIG. 41. Interpretive drawing of figure 40. ×2650. FIG. 42. Polar aspect of metaphase showing ring of ca. 8 chromosomes. ×2650. FIG. 43. Lateral aspect of polyploid nucleus in early anaphase; compare width of spindle with that of division figure in figure 42. ×2650. FIG. 44. Two haploid nuclei at late anaphase, portion of spindle visible in nucleus at the right. ×2650. FIG. 45. Cell with 2 nuclei (only 1 in focus), ca. 8 chromosomes visible in late prophase. ×2650. Fig. 46. Cell showing polyploid nucleus at anaphase. ×2650. FIG. 47. Interpretive drawing of figure 46. ×2650. FIG. 48. Two polyploid nuclei in metaphase, plates of chromosomes slightly tilted and distorted. ×2650. FIG. 49. Interpretive drawing of figures 48. ×2650. FIG. 50. Cell with 4 polyploid nuclei at metaphase, the lower 2 of which are seen as flat plates containing many chromosomes; upper 2 not in focus. ×2650. FIG. 51. One focus of cell whose 16 nuclei are dividing to form 32. Note small ("reduced") number of chromosomes exhibited by division figures. ×2650. FIG. 52. Actively growing vegetative cells immediately following transfer to Knop agar and prior to exposure to a light intensity of 800 foot-candles. ×530. FIG. 53. Living cells from the same plate as those shown in figure 52 after growth at 800 foot-candles of light intensity for 12 hours. ×530. FIG. 54. Living cells from the same plate as those shown in figures 52 and 53 after growth at 800 foot-candles of light intensity for 15 hours. Cells in all stages of division to produce daughter cells. ×530. FIG. 55. Nucleus of germinating zygote approaching synizesis with chromatinic threads oriented; note large nucleolus. ×11,000. FIG. 56. Post-synizetic nucleus of germinating zygote, chromatinic threads loosening. ×11,000. FIGS. 35, 36, 39–51. Aceto-carmine preparations. FIG. 37. Azure A preparation. FIGS. 55, 56. Iron-alum haematoxylin preparations.

plication was shown to be a light effect, and not a genetically controlled phenomenon.

At first metaphase in such enlarged cells of *C. reinhardti* and *C. chlamydogama*, there proved to be no disturbance of chromosome number, that is, 8 chromosomes could be seen as a hollow ring in polar view at any given metaphase. However, in cells of *C. eugametos* and *C. moewusii*, the first metaphase of such large cells always exhibited more than 8 chromosomes. In such cells, approximately 16, 32 or 64 were observed. Subsequent division of the original large cell to form 2, 4, 8, 16 or 32 daughter cells invariably was accompanied by a reduction in chromosome numbers (fig. 51). This reduction occurred regardless of whether daughter cell formation occurred at a light intensity of 800 foot-candles, one of 100 foot-candles, or in total darkness.

Although a direct correlation was not always observed between cell size and the degree of polyploidy, it was generally true that large cells produced at high light intensities were polyploid, and that small cells were not. However, some cells which were relatively quite small were seen to exhibit more than 8 chromosomes at first division (figs. 46, 47).

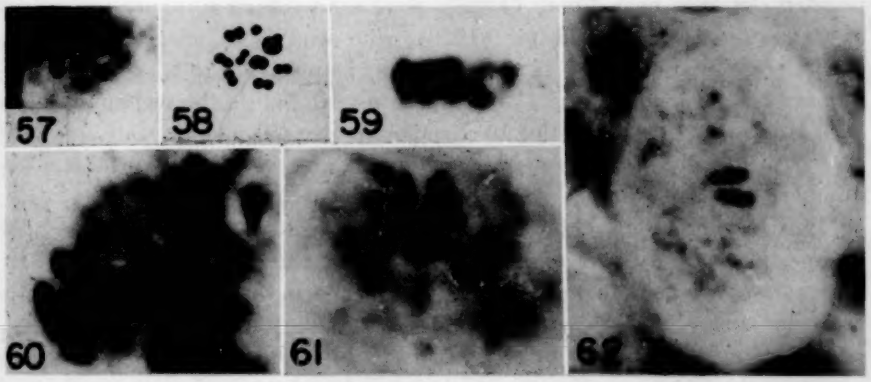
The phenomena of endomitosis and somatic reduction as they apparently occur in vegetative cells of *C. eugametos* and *C. moewusii* are illustrated diagramatically by figure 1. Upon a given plate of Knop agar, when maintained at 800 foot-candles of continuous illumination, these phenomena are accomplished repeatedly by the enlargement of small cells initially spread upon the surface of the medium, production by these of haploid daughter cells, their enlargement again accompanied by endomitoses, and so on, until crowding of cells upon the plate obviates a further manifestation of these phenomena. Observation of such a plate upon which a solid growth of cells is seen, usually evident by 72 hours if the initial inoculum was fairly heavy, reveals the fact that all cells are small in size. If these, in turn, are spread upon another plate, the increase in cell size and polyploidy again become evident.

Meiosis. Details of the meiotic process as studied in germinating zygotes of each species proved to be essentially similar; because preparations of *C. chlamydogama* exhibited the greatest number of critical division stages and were in general more revealing than those of the other species, the process will be described in detail as it occurs in that organism.

During the time between formation of the mature zygote and initiation of prophase of the first meiotic division, a thick wall develops, from which the zygotic protoplast appears to be shrunken in fixed and stained preparations (fig. 18). This shrinkage is no doubt due to the slow penetration of the fixative through this wall, as living zygospores do not exhibit this characteristic. The zygospore wall also proves to be impermeable to stains.

With the initiation of meiosis, however, a change in permeability occurs, and the nuclear stains used in this study enter the cell readily. It is not until after the formation of 4 daughter cells, however, that fast green counterstain is able to penetrate the wall readily.

At early prophase, a number of chromatinic bodies may be seen within the nucleus, these being connected by strands (fig. 18). This material becomes more and more condensed (fig. 19), and eventually a synizesis-like pattern is evident (figs. 20, 55, 56). Further condensation results in a number of bodies many of which are connected (fig. 21). At a slightly later stage, these bodies, which now number approximately 16, no longer give evidence of being connected (fig. 22). However, a stage next occurs



FIGS. 57–61. *Chlamydomonas chlamydogama.* FIG. 62. *Chlamydomonas eugametos.* FIG. 57. Diakinesis in germinating zygote. ×5500. FIG. 58. Interpretive drawing of figure 57. FIG. 59. Metaphase of first meiotic division of zygote, lateral aspect. Four pairs of chromosomes separating with 4 others behind (not visible). Separating pair at right slightly crushed apart from the others. ×11,000. FIG. 60. Late prophase of second meiotic division in germinating zygote. Note ca. 8 chromosomes and nucleolus in nucleus at left. ×11,000. FIG. 61. Anaphase of first meiotic division, separating rings of 8 chromosomes slightly crushed. See figure 27 for interpretive drawing. FIG. 62. Anaphase of first meiotic division showing 2 separating rings of ca. 8 chromosomes in lateral aspect. See figure 34 for interpretive drawing. All figures from iron-alum haematoxylin preparations.

in which bodies are somewhat larger and clearly paired, this stage being diakinesis (figs. 23, 57, 58). After this, a metaphase ring of 8 double chromosomes become oriented upon a spindle whose fibers are clearly evident with the technique employed (figs. 24, 59). Anaphasic separation reveals the movement of 8 chromosomes toward each pole (figs. 25–28, 61), the telophase stage also revealing approximately this same number (fig. 28). Two daughter cells form within the original zygote wall (fig. 29), the second meiotic division occurs (figs. 30, 60) and the 4 daughter cells within the original wall (fig. 31) constitute the final meiotic products (in *C. chlamydogama*).

The patterns of meiosis as observed in *C. reinhardti, C. eugametos* and *C. moewusii* appear to be identical to that described for *C. chlamydogama.* Figures 32–34 and 62 show representative stages of meiosis as it was observed in these species.

Spindle fibers were clearly observed in several mitotic figures of all species when cells were stained by the modified Cave and Pocock aceto-carmine method and in meiotic figures of cells prepared by the iron-alum haematoxylin technique. No centrioles were observed in cells of any species.

Discussion. The fact that the haploid chromosome number of all 4 species of *Chlamydomonas* studied proved to be n = 8 suggests the possibility that all species of the genus exhibit a relatively low number. It has been pointed out that both Bêlaŕ (1926), working with an unidentified species of *Chlamydomonas,* and Kater (1929), who studied *C. nasuta,* reported a chromosome number of 8 in each species. Dangeard (1899) reported the number of *C. variabilis* and *C. dilli* to be n = 10. While the writer disagrees with Moewus' (1936) count of 10 chromosomes in *C. eugametos,* it is evident that both it and *C. paupera,* which he states are interfertile, exhibit a low number.

It is felt that Schaechter and DeLamater's (1955) counts of 36 ± 2 for *C. moewusii,* 36 ± 4 for *C. eugametos,* 18 ± 2 for *C. reinhardti* and 16 ± 1 for *C. dysosmos* are open to question. In the first place, these authors apparently were not aware of the light effect described above which results in temporary polyploidy in cells of *C. moewusii* and *C. eugametos.* While they do not state the light intensity which they employed, their figures of preparations made from cells of these two species give every indication that they made their counts from such polyploid cells. A second factor which may have given rise to error in their work lies in the fact that nuclei which are in earlier prophase exhibit a number of chromatinic bodies which exceeds that of the chromosomes at metaphase and anaphases, a phenomenon which was also observed by Dangeard (1899) and Kater (1929). The technique of Schaechter and DeLamater consisted of squashing cells apparently before they examined them microscopically; in such preparations, it would be difficult to determine with certainty the actual stage of division of any given nucleus. Furthermore, squashing may tend to separate chromatids at late prophase or metaphase, and it has been pointed out by Steere, Anderson and Bryan (1954), in their work on the chromosomes of mosses, that in cells which have been strongly flattened, first anaphase or second metaphase plates may flow together and appear as a single figure, thus presenting a misleading picture. While the squash technique is a standard procedure in cytological work, counts made in this fashion in cells of *Chlamydomonas* are somewhat suspect for the reasons given.

From a critical examination of Schaechter and DeLamater's figures, it

seems evident that a combination of these factors may be responsible for their interpretations of chromosome number in *C. moewusii* and *C. eugametos*. Their photographs and interpretive drawings of the chromosomes of *C. reinhardti* are somewhat more difficult to reconcile with counts which were made in connection with the present study, but there seems to have been a certain degree of crushing apart of chromatids. It is quite possible that their two photographs (18, 20) of *C. reinhardti* chromosomes, presenting 18 and 19 bodies respectively, represent late prophase figures in which full condensation had not occurred in the case of some of the bodies, while others were sufficiently advanced that the squashing apart of chromatids could occur. The writer has observed many cells of *C. reinhardti* at late prophase in which 10–12 bodies could be seen; the right-hand nucleus in figure 36 is an example of this.

In their observations on the meiotic chromosomes of *C. moewusii*, Schaechter and DeLamater (1954) reported "the number of loops and of diakinesis-like configurations," in the first meiotic division, to be "in the order of 8 to 10." However, they later (1956) suggest "the possibility that the mechanism of meiosis in this organism is of some aberrant form" since the counts of 8 to 10 did not correspond to their report of 36 ± 2 in vegetative cells of this species which they had made previous to studying meiosis.

Krauss and Wetherell (1956) have reported the chromosome number of *C. reinhardti* to be 16, and they have published photographs described as "metaphase," showing approximately this number of bodies. However, in view of the spherical form of the figure, which required photographs at three focal levels to include all the chromatinic bodies, the nucleus photographed seems to be in prophase, and it is probable that they counted bodies which had not yet condensed into chromosomes.

The only other case of which the writer is aware in which a species of *Chlamydomonas* has been reported as exhibiting more than a haploid chromosome number of 10 is Dangeard's (1899) count of 30 in *C. monadina*. It is possible that this species is subject to the same light effect as *C. eugametos* and *C. moewusii*, reported in this paper, and that this effect escaped Dangeard. One of his figures shows a second division in a vegetative cell in which one of the nuclei is in early anaphase, as seen in lateral aspect. The other nucleus apparently is in metaphase and exhibits a polar aspect of a flat plate of chromosomes. These figures are strikingly similar in appearance to comparable stages in *C. eugametos* and *C. moewusii* when cells of these two species are still polyploid.

In the transformation from small to large cells under the influence of high light intensities, the species of *Chlamydomonas* studied show a remarkable parallel to the behavior of *Chlorella ellipsoidea*, where "dark

cell'' and ''light cell'' forms have been described (Tamiya, Iwamura, Shibata, Hase and Nihei 1953). It seems likely that the mechanisms responsible for this parallel behavior are the same. An extension of the PNA and DNA determinations of Iwamura (1955) on *Chlorella ellipsoidea* to *Chlamydomonas eugametos* and *C. moewusii*, especially, should go far toward revealing the degree of similarity involved.

That light may be an important factor in stimulating or inhibiting degree of polyploidy is clear from the results of v. Witsch and Flügel (1951). Dividing nuclei of callus produced by mesophyll cells of short day plants of *Kolanchoë Blossfeldiana* exhibited chromosome numbers up to 32n (= 544!) while comparable nuclei of long-day plants showed much lesser degrees of polyploidy.

It is of interest that in haploid cells of *C. eugametos* and *C. moewusii* the 8 chromosomes are characteristically arranged in a ring at metaphase (fig. 42) while polyploid nuclei exhibit flat plates of chromosomes (fig. 50). Fankhauser and Humphrey (1950) have shown that this same pattern of chromosomal behavior appears in polyploid nuclei of the axolotl, that is, metaphase chromosomes of normal diploid cells appear in a ring, while those of polyploid cells become crowded into the central portion of the equatorial plate.

Although it is possible to describe the details of chromosomal behavior during somatic reduction in those organisms where the chromosomes are of sufficient size to allow it, this has not been possible for *C. eugametos* and *C. moewusii* in the present study due to the extremely minute size of the chromosomes. It is to be hoped that techniques will be developed which will render a closer study possible, but at the present time these details cannot be described. The meiotic process seems clearly to follow a classical pattern.

SUMMARY

1. Four species of *Chlamydomonas, C. eugametos* Moewus, *C. chlamydogama* Bold, *C. reinhardti* Dangeard and *C. moewusii* Gerloff, were studied cytologically and compared. Particular study was devoted to mitosis and meiosis. These processes proved to be essentially similar in all 4 species and to follow the classical patterns observed among plants. No dissimilarities were noted in mitosis as it occurs in these organisms and the pattern which has been described for other species of *Chlamydomonas* by earlier investigators.

2. The haploid chromosome number was found to be 8 for all 4 species; this constitutes a much lower count than those reported by recent workers for *C. eugametos, C. reinhardti* and *C. moewusii.*

3. It was found that when vegetative cells of *C. eugametos* and *C. moewusii* are grown at a light intensity of 800 foot-candles, temporary

polyploidy is exhibited by certain cells. Somatic reduction, the exact mechanism of which was not determined, eventually results independently of light.

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BULLETIN OF THE TORREY BOTANICAL CLUB
Vol. 85, No. 3, pp. 179–181 MAY, 1958

PIPTOCHAETIUM AND PHALARIS IN THE FOSSIL RECORD[1]

A. A. BEETLE

Identification of grasses from fossil floras is so difficult that many published findings are erroneous (Elias 1942). Thus *Poa*-like remains turn out to be horsetail, and specimens identified as *Stipa* turn out to be mountain-mahogany fruits.

Perhaps then, the identification of fossil grasses is only a guessing game, but if so it can be intriguing and should not be abandoned. Eventually a sufficient number of correct guesses can lead to important conclusions concerning the grasslands.

MacGinitie (1953) recently reviewed the work since 1874 covering the fossil plants of the Florissant Beds of Colorado. From this flora two grasses have been described. The first, *Melica primaeva,* is rejected by MacGinitie because "the specimen is too poor for identification," the second, originally described as *Muhlenbergia florissanti,* is transferred to *Stipa.* Two new dispositions are suggested for these grasses as follows:

Phalaris primaeva (C. T. and B. B. Brues) new combination

Melica primaeva, C. T. and B. B. Brues, Bul. Wisc. Nat. Hist. Soc., **6**: 170, 1909.

Piptochaetium florissanti (Knowlton) new combination

Muhlenbergia florissanti, Knowlton, Proc. U. S. Nat. Mus., **51**: 250, 1916.

Stipa florissanti (Knowlton) MacGinitie, Carnegie Inst. of Wash. Public. **599**: 91, 1953.

The first means of identifying a grass in any fossil flora is by direct comparison, as is done with living plants. However, the chances of error can be greatly reduced if one employs the evidence of inference, building a picture of what could, and what could not be present. When this is done, an identification outside this body of reference would not be impossible but an identification within would greatly strengthen the whole.

We are told by MacGinitie through interpretations from other more readily identified material that the grass to be present in the flora of the Florissant Beds must:

(1) Date on the North American continent back at least to Oligocene.
(2) Must be adapted to arid or semi-arid conditions.
(3) Be a North American native and probably within limits a North American endemic.
(4) Have its principal distribution at present southward.

[1] Published with approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 99.

The grass genera, at least some of whose members fulfill the above requirements, are (Hitchcock 1951):

Aveneae: *Dissanthelium*

Pappophoreae: *Cottea, Pappophorum, Enneapogon, Scleropogon*

Agrostideae: *Polypogon, Lycurus, Muhlenbergia, Sporobolus, Blepharoneuron*

Stipeae: *Piptochaetium, Stipa, Aristida*

Zoysieae: *Hilaria, Aegopogon*

Chlorideae: *Microchloa, Willkomia, Schedonardus, Chloris, Trichloris, Bouteloua, Cathestecum, Buchloë*

Eragrosteae: *Tridens, Leptochloa, Tripogon, Trichoneura, Blepharidachne*

Phalarideae: *Phalaris*

Paniceae: *Trichachne, Eriochloa, Brachiaria, Paspalum, Panicum, Oplismenus, Setaria*

Andropogoneae: *Imperata, Andropogon, Trachypogon, Elyonurus*

Fossils of any of these might be expected to occur in the Florissant Beds. This large number of possibilities emphasizes both the poor chances of grasses to enter the fossil record and the richness and diversification of the grass family in Tertiary time.

Along with the original description of *Melica primaeva* (Brues and Brues 1909) there is an excellent figure of the fossil impression on which *Melica primaeva* is based. Here the interpretation of this figure by Brues and Brues is challenged. Examination shows a number of significant facts are evident:

(1) Lack of awns
(2) Lack of prominent veins
(3) Comparative great width in relation to length of glume, lemma, and palea
(4) Comparative size of glume to lemma
(5) Comparative size of lemma to palea
(6) Paired grouping of florets

Examining the list of grass genera above it would appear that only *Phalaris* (Phalarideae) fulfills all of these requirements. Living (and presumably related) *Phalaris* today include *P. angustata, P. Lemmoni,* and *P. californica.* The illustrations of Riecken (1929) of *Torresia odorata* (now *Hierochloë*) are especially suggestive of the illustration of Brues.

MacGinitie (1953) has clearly illustrated the fossil material on which *Muhlenbergia florissanti* was based. The figure shows:

(1) A well-developed awn, weakly flexuous
(2) A callus
(3) One-flowered spikelets
(4) Some dorsal compression of the spikelet

This grass is of the Stipeae, and if the unbranched awn rules out *Aristida* then the choice falls to either *Stipa* or *Piptochaetium* (cf. *P. fimbriatum*). The dorsal compression, weakly flexuous awn, and the rather abrupt point of attachment of awn and fruit all suggest *Piptochaetium* only, while all other points are equally suggestive of either *Stipa* or *Piptochaetium*. The illustration of Vasey (1891) of *Piptochaetium fimbriatum* is especially suggestive of that of MacGinitie.

The presence of *Phalaris* and *Piptochaetium* in Colorado in late Eocene and early Oligocene would not be at all surprising. Both genera are well-known southward to South America. Neither is known northward (except for the Pleistocene dispersal of *Phalaris arundinacea*). They appear to have been at the time of their preservation near the northward limit of a Cretaceous dispersal of grasses which started in the Old World, crossed Antarctica, and then spread northward through the Americas. Some representatives of the species in this dispersal completed their northward spread in Central North America during or before the close of Eocene and formed the basis for grasses in the Florissant Beds.

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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 85, No. 3, pp. 182–187 MAY, 1958

VITAMIN REQUIREMENTS OF THREE LICHEN FUNGI[1]

MASON E. HALE, JR.

The vitamin requirements of the fungal components of lichens are almost completely unknown. None of the physiologists prior to 1930 studied vitamins, for these compounds were not available in pure form at that time. Quispel (1946) cultured in synthetic media several parasymbiotic fungi associated with free-living algae on trees and the slow growing lichen fungus *Xanthoria parietina*. He found some evidence of deficiencies for thiamine. Zehnder (1949) studied the growth requirements for a number of lichen fungi on purified agar to which various vitamins were added. He discovered only five species with an appreciable growth rate and only one of these, *Lecanora saxicola*, a crustose saxicolous species, grew better on a medium with thiamine added than on vitamin free agar. Misch (1953) made a broad study of the nutrition of the lichen fungus *Umbilicaria papulosa*, which required thiamine for optimal growth but gave rather low yields under the conditions studied.

The writer has isolated about 20 different lichen fungi from ejaculated spores. Three isolates grew exceptionally rapidly on malt extract-yeast extract agar, forming colonies nearly one centimeter in diameter after a month. These three, selected for a careful study of vitamin requirements, were *Acarospora fuscata* (Nyl.) Arn., a crustose discomycete in the family Acarosporaceae (Hale, no. F24, on exposed sandstone, Dolly Sods, Randolph Co., W. Va.), *Buellia stillingiana* Steiner, a crustose discomycete referable to the Patellariaceae and with a conidial stage equal to *Sporidesmium folliculatum* (Corda) Mason and Hughes (Hale 1957) (Hale, no. Fla, on bark of *Acer rubrum*, near Cheat Bridge, Randolph County, W. Va.), and *Sarcogyne similis* Magn., an endolithic crustose discomycete in the Acarosporaceae (Hale, no. Fl, on sandstone, near High View, Hampshire County, W. Va.). *Acarospora fuscata* and *Sarcogyne similis* were identified by A. H. Magnusson, and *Buellia stillingiana* by H. A. Imshaug. Specimens of the symbiont lichens are preserved in the Smithsonian Institution, and subcultures of the isolated lichen fungi are maintained at the Centraalbureau voor Schimmelcultures, Baarn.

Media. The basic synthetic medium developed by Lilly and Barnett (1951, p. 427) was modified as follows: Carbohydrate (10 g.); KH_2PO_4 (1 g.); $MgSO_4 \cdot 7H_2O$ (.5 g.); ammonium tartrate (2 g.); Fe^{+++} (0.2 mg.); Zn^{++} (0.2 mg.); Mn^{++} (0.1 mg.); $CaCl_2$ or $CaCO_3$ (0.2 g.); and distilled

[1] The experimental work was conducted in the Department of Plant Pathology, West Virginia University. The aid and advice of Dr. V. G. Lilly is gratefully acknowledged.

water (1000 ml.). Four vitamins were added at the following rates per liter: Thiamine (100 μg.); biotin (5 μg.); pyridoxine (100 μg.); and inositol (5 μg.).

Glucose was used as the carbon source for *S. similis*, while the sugar alcohol mannitol was employed for *A. fuscata* and *Buellia stillingiana*. Several preliminary experiments were run without added calcium. Under these conditions *B. stillingiana* did not sporulate but produced abundant white mycelium. Later $CaCl_2$ was added and, surprisingly, abundant sporulation occurred between pH 3.8 and 6. The final yield was significantly greater than in the control without calcium. It was concluded tentatively that this species is partially deficient for calcium. Near the end of preliminary runs, $CaCO_3$ was accidentally substituted for $CaCl_2$ with the unexpected results that the lag phase, ordinarily about 10 days, was shortened to 4–6 days, sporulation was completely inhibited, and the yield of mycelium slightly exceeded that with $CaCl_2$ added. The final experiments therefore were run with $CaCO_3$ added for *B. stillingiana* and $CaCl_2$ for the other two species which actually were not affected by the omission of calcium.

A vitamin free medium was prepared according to directions in Lilly and Barnett (1951, p. 421) using Norite. After appropriate vitamins and trace elements were added to the purified stock solutions, the pH was adjusted to 5–6 and aliquots of 25 ml. were measured into 250 ml. erlenmeyer flasks. All media were autoclaved for 15 minutes at 15 pounds steam pressure. Mycelium for stock liquid cultures was macerated in a sterile Waring blendor and 4–6 drops of the suspension were added to each flask as inoculum. *A. fuscata* and *S. similis* were incubated at a constant temperature of 20° C, *B. stillingiana* at 25° C with all cultures receiving 12 hours of diffuse artificial light and 12 hours of darkness each day.

Results. Growth patterns on the complete medium. All three fungi had typically shaped growth curves (figs. 1–3) on the basal medium to which the four vitamins were added. The slowest grower, *A. fuscata*, had a lag phase of about three weeks, then a steady and rapid increase in growth until a maximum of about 100 mg. of mycelium per flask was reached in seven weeks. This fungus formed compact, straw-colored, submerged balls about one centimeter in diameter which fragmented with difficulty. The initial pH of 5.2 dropped to a minimum of 3.5–3.7 at seven weeks, then began to rise as autolysis commenced.

Sarcogyne similis had a similar growth pattern. After a lag phase of about two weeks, a growth peak was reached in five weeks with a maximum yield of 110 mg. per flask. The mycelium was pure white, flocculent and fragmented easily. After several weeks the mycelium would rise somewhat to the surface of the medium. An initial pH of 4.8 dropped to 3.3–3.5 in five weeks, and then rose.

Buellia stillingiana had a lag phase of less than one week, which was followed by a very rapid rate of growth to a sharp peak of nearly 130 mg. of mycelium in three weeks, followed by rapid autolysis. The mycelium was pure white, slimy, and usually submerged. The initial pH of 5.9 fell to 3.8 at the growth peak, then increased to nearly 5.2 as autolysis began.

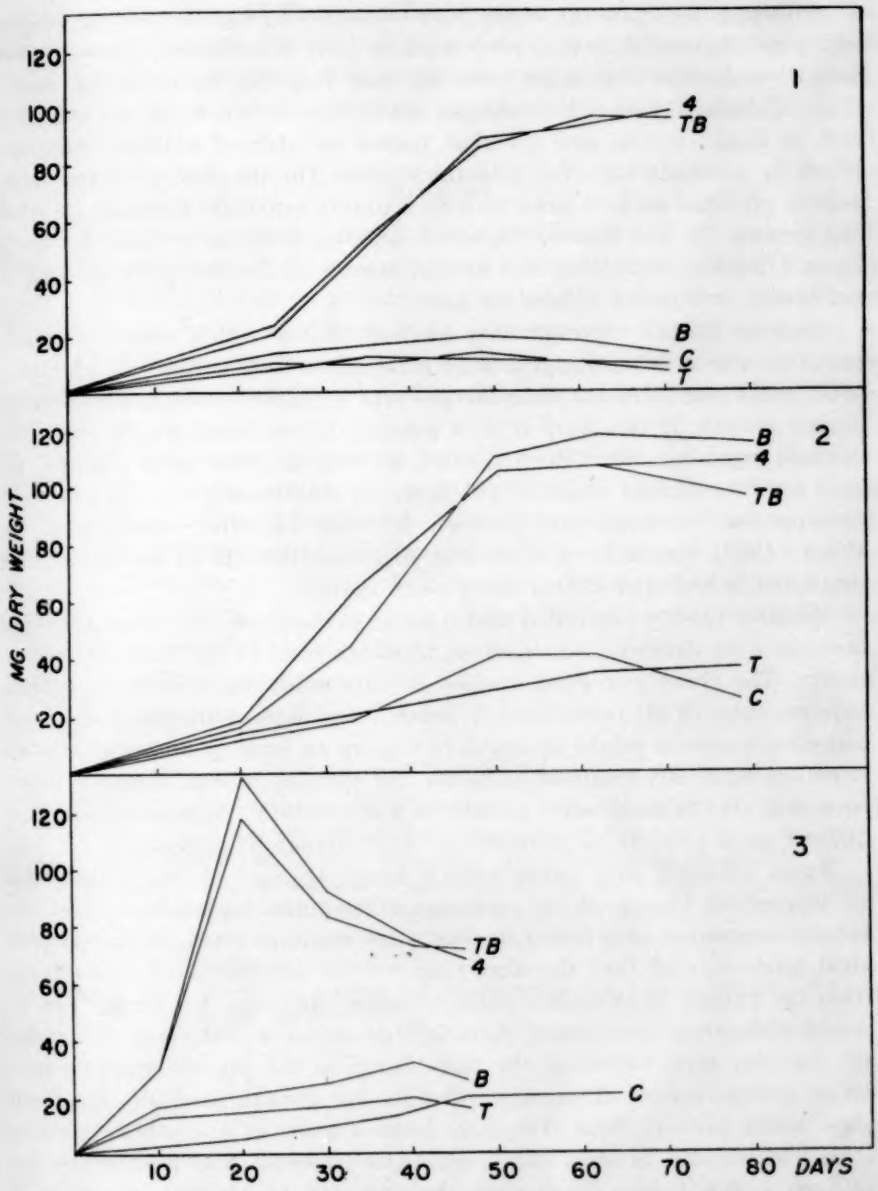
These three fungi approach the growth rates and yields of some saprophytic discomycetes, such as *Helotium epiphyllum* or *Chlorosplenium aeruginosum* (Oed.) Fr. on similar media. Unfortunately this rapid growth is exceptional, for most lichen fungi studied thus far produce at most 10–20 mg. of mycelium after months of cultivation.

Growth on incomplete media. The three isolates were cultured in four other media as a test for vitamin requirements: A vitamin free control, one with thiamine added, one with biotin added, and one with both thiamine and biotin added. The resulting growth curves are summarized in figure 1–3. It is obvious that none of these fungi require an exogenous source of inositol or pyridoxine, since media with only thiamine and biotin added support as much growth as the complete medium. On the other hand, the omission of biotin from the medium reduced the growth of all three species. A deficiency for biotin has not been reported or proved for any lichen fungi. While *S. similis* required only biotin for maximum growth, *A. fuscata* and *B. stillingiana* also require the addition of thiamine to match growth on the complete medium.

The curves for the growth of *A. fuscata* and *B. stillingiana* on the controls reflect a total deficiency for thiamine and biotin. The small yields of 10–20 mg. from the control flasks can probably be attributed to contaminating vitamins from the rather large inoculum. In any event, the two isolates did not give any indication of becoming adapted to growth in a vitamin free medium after more than two months. *Sarcogyne similis* without added vitamins produced about one-third as much mycelium as on the complete medium. This isolate would be classified as partially deficient for biotin.

The absence of essential vitamins had no effect on the gross appearance of cultures of *S. similis* or *B. stillingiana*. *Acarospora fuscata*, however, developed a brownish purple pigment on media lacking biotin or thiamine, apparently the same as the pigment that gives the characteristic brown color to the symbiont lichen. Its structure is unknown. No reproductive structures other than the conidia of *B. stillingiana* were found in the cultures, nor were any lichen acids demonstrated even though the symbiont thallus of *B. stillingiana* contained atranorine and norstictic acid.

Discussion. It is exceedingly fortunate that three such rapidly growing lichen fungi were isolated. This factor alone made possible an analysis of the vitamin requirements of these species with accepted physiological techniques.



FIGS. 1–3. Growth curves for three lichen fungi in five different media. FIG. 1. *Acarospora fuscata.* FIG. 2. *Sarcogyne similis.* FIG. 3. *Buellia stillingiana.* The lines connect points representing the average dry weight of mycelium from two 250 ml. flasks with 25 ml. of medium per flask. C represents the growth in a control without added vitamins; T, thiamine added; B, biotin added; TB, thiamine and biotin added; and 4, thiamine, biotin, inositol, and pyridoxine added.

Although they have obviously been hampered by slowly growing isolates and poor choices of media, past workers have nonetheless drawn rather broad conclusions that some botanists may consider valid for all lichen fungi. Zehnder (1949), for example, states that lichen fungi do not grow well in liquid media, and for that reason he utilized an agar substrate which is unsatisfactory for vitamin studies. On the contrary, the three isolates reported on here grew well on a purely synthetic medium. In other experiments by the writer, *Parmelia isidiata, Graphis scripta, Lecanora varia, Cladonia cristatella,* and several species of *Lecidea* grew as well, if not better, in liquid media as on agar.

Quispel (1946) reported that his isolates, of which only *Xanthoria parietina* was a lichen fungus, were little affected by initial pH (between pH 2 and 6) and did not alter the pH of a synthetic medium significantly during growth. If this were true in general, lichen fungi would indeed be unusual organisms. Our three isolates, as well as other species tested, all have a rather narrow range of pH in which maximum growth is achieved, ranging for the most part between 4.5 and 5.5 after autoclaving. As Misch (1953) found for *Umbilicaria papulosa,* the pH of the medium is continuously changed during the growth cycle.

Zehnder (1949) concluded that none of his isolates, excepting *Lecanora saxicola,* were deficient for thiamine, although most of his fungi grew very slowly. The three unrelated species in this study have definite vitamin requirements. In all probability if lichen fungi were more easily cultured, many more species would be found to require an exogenous source of some vitamins, especially thiamine or biotin, for optimal growth. Unless a lichen fungus is able to make some growth in a synthetic medium, however, it is difficult or impossible to demonstrate any vitamin requirements.

From his work with parasymbiotic fungi, Quispel (1946) propounded an interesting theory of an exchange of essential metabolites—that the fungal component of a lichen derives some vitamins from the autotrophic algal partner, and that the alga may receive ascorbic acid in exchange from the fungus to stimulate photosynthesis. Although his theory has received widespread acceptance, there is little evidence that fungi do synthesize ascorbic acid. Certainly the three fungi in the present study require an exogenous source of certain vitamins for growth, and the symbiotic algae could provide them. Yet these isolates grow in a synthetic medium almost as rapidly as some saprophytic discomycetes, which suggests that at least a few lichen fungi could be expected to survive in nature as saprophytes without an associated alga and derive vitamins and nutrients from dead leaves or wood. Symbiosis may therefore represent only one phase in the life cycle of some lichen fungi. This possibility has received some support from the recent discovery (Hale 1957) that the conidial

stage of one isolate of *Buellia stillingiana* is apparently identical to the imperfect fungus *Sporidesmium folliculatum*, a common saprophyte on wood. Until more is known of the basic nutrition of lichen fungi in synthetic media, it is premature to speculate on the nature of symbiosis of lichen fungi and algae.

SUMMARY

1. Three crustose lichen fungi, *Acarospora fuscata* (Nyl.) Arn., *Buellia stillingiana* Steiner, and *Sarcogyne similis* Magn., were isolated from the symbiont lichen thallus by germinating ejaculated ascospores. All isolates grew comparatively rapidly on malt extract-yeast extract agar and in synthetic liquid media. No ascocarps or lichen acids were produced in pure culture. *Buellia stillingiana* formed conidia when $CaCl_2$ was added to the medium.

2. Vitamin requirements were tested by culturing the lichen fungi in five different media; namely, a vitamin free control, thiamine added, biotin added, both thiamine and biotin added, and four vitamins, thiamine, biotin, inositol, and pyridoxine, added. None of the isolates required an exogenous source of inositol or pyridoxine. *Acarospora fuscata* and *Buellia stillingiana* were totally deficient for both thiamine and biotin. *Sarcogyne similis* was partially deficient for biotin only.

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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 85, NO. 3, PP. 188–196 MAY, 1958

HYGROLEJEUNEA ALASKANA SP. N., A CRITICAL ENDEMIC OF NORTHERN ALASKA

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The genus *Hygrolejeunea* (Spruce) Schiffner originally included 13 species, all from tropical America. By 1923 Stephani had increased the number to 106 species, and had extended the range of the genus through the tropical portions of the globe: with 13 African, 42 Neotropical, 46 Oceanic-tropical Asian, and 4 Australian species. However, Eifrig (1937) found that of the 46 species ascribed by Stephani to East Asia and Oceania, only 21 were valid species of *Hygrolejeunea;* 9 were found to represent species of *Taxilejeunea*, while the other 16 were synonyms. Furthermore, Stephani described several species of "*Lejeunea*" that are identical or close to *Hygrolejeunea discreta* (Ldb.) St., while *Taxilejeunea grandistipula* St. proved to be identical with *Hygrolejeunea molkenboeriana* St. Stephani also described plants belonging to *Euosmolejeunea* as *Hygrolejeunea* (Eifrig 1937).

From this history of past confusion, it is evident that *Hygrolejeunea* has not been sharply separated from several other genera of Lejeuneaceae, chiefly *Lejeunea* s. str., *Taxilejeunea*, and *Euosmolejeunea*. In fact, the genus is one of the many "uncomfortably vague" genera of Schizostipae. Spruce (1884, p. 231), in his original description of the genus, compares *Hygrolejeunea* only with *Euosmolejeunea*. It shares with the latter a number of superficially similar features—large, often cordate underleaves; deflexed leaf apices; a similar growth pattern and habitus—but is not closely allied to it. *Hygrolejeunea* can be separated from *Euosmolejeunea* at once by (a) the proximal position of the hyaline papilla of the lobule, with respect to the apical tooth; (b) the much more leptodermous and pellucid, delicate cells; (c) absence of any odor, and, associated, smaller and less persistent oil-bodies; (d) laxer, looser habitus; (e) the much paler, more whitish color, without the olive or greyish tinge of *Euosmolejeunea;* (f) shiny texture.

In all these characters, *Hygrolejeunea* much more closely approximates *Lejeunea* and *Taxilejeunea*. It is with these, indeed, that the most immediate points of contact exist. There is, in fact, no absolute basis for the separation of *Hygrolejeunea* from either of these genera, and a good case could be presented in favor of combining it with either (or both) of them. However, the most "typical" species of the genus, such as the genotype and *H. spongia*

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(Spr.) and *H. devoluta* (Spr.), stand isolated from both of these allied genera in that the gynoecia are almost uniformly situated in the fork of a dichotomy (the gynoecia develop paired, and usually sterile, innovations). In this respect our single species is quite typical.[3]

Some species of *Hygrolejeunea* approach *Taxilejeunea* very closely in most respects, in, for instance, (a) the acute or apiculate, ovate-cordate leaves; and (b) the cordate or subcordate, extremely large and densely shingled underleaves. The separation of such species from *Taxilejeunea* is very difficult, especially since many plants have a single subfloral innovation, which may again be fertile. In such cases, there is little or no difference from the less specialized species of *Taxilejeunea*.[4]

In a minority of species, the underleaves are hardly imbricate, scarcely cordate at base, smaller than the lateral leaves, and bifid to one-third. In such species, an approach exists to *Lejeunea* subg. *Lejeunea* (in which, however, there is normally only one subfloral innovation). Our single species, which approaches *Lejeunea* in some of these vegetative features, has the paired gynoecial innovations characteristic of many species of *Hygrolejeunea*.

Although there is nothing absolute about the number of gynoecial innovations, this feature does appear to enable us to segregate three distinct (if not sharply defined) groups in this difficult complex of genera. The writers would suggest the use of the gynoecial innovations as a primary character, supplemented by others, resulting in the following separations:

1. Gynoecia (except sometimes the primary one) almost constantly with a single innovation (which often is again floriferous) 2
2. Gynoecial innovation soon floriferous, subtended by a short, repeatedly floriferous innovation (the gynoecia appearing approximated and pseudolateral, all on the same side of the axis, forming thus a sympodial system); leaves commonly

[3] Spruce (1884, p. 230) clearly emphasizes this as one of the generic characters of *Hygrolejeunea*, stating "♀ saepissimo dichotomis," and again (p. 231) "ramis ♀ persaepe dichotomis." He clearly illustrates this condition for *H. devoluta* (Spr.). Unfortunately, Schiffner (1893, p. 125) states that the gynoecium is pseudolateral, and Vanden Berghen (1948, p. 27) follows him in assigning only 1 subfloral innovation to this genus.

[4] Eifrig (1937) has also emphasized that the boundaries between *Taxilejeunea* and *Hygrolejeunea* are not sharp, and found it necessary to move several species that Stephani had ascribed to *Hygrolejeunea*, from this genus to *Taxilejeunea*. Indeed, on the basis of the study of the Indomalayan species, Eifrig concluded that *Hygrolejeunea* was a "schlecht begrundete Gattung." Eifrig found that on careful study those Indomalayan species with divided underleaves were referable usually to *Taxilejeunea*. He implies that the schizostipous species of *Hygrolejeunea* may prove partly referable to *Lejeunea*. The possibility therefore exists that *Hygrolejeunea* will be found to be a relatively small genus, embracing mostly holostipous species. Unfortunately, *H. cerina*, previously suggested as genotype, represents a plant showing the facies of *Taxilejeunea* (judging from the two available collections), and is hardly representative of *Hygrolejeunea* in Eifrig's sense.

apiculate or acute, many with 1-several preapical teeth; underleaves often *large, frequently cordate at base,* usually wider than long and shallowly bilobed; cells normally with large trigones and intermediate thickenings. Perianth often variously specialized (beak and/or keels reduced; keels armed, etc.). *Taxilejeunea*

2. Gynoecial innovations sterile, or fertile after the development of several pairs of leaves; not, or only exceptionally, with the innovations repeatedly fertile, thus without formation of sympodial clusters of gynoecia; leaves usually rounded at apex and entire, without teeth; underleaves small to very small, exceptionally subcordate, usually bifid to middle or deeper, usually as long or longer than broad; cells usually with minute trigones, and with intermediate thickenings absent or scarce. Perianth with keels distinct (except sometimes the antical), beak distinct, the keels smooth or crenulate ..... *Lejeunea*

1. Gynoecia usually (or frequently) in the fork of a dichotomy, i.e., with 2 innovations; innovations usually sterile (rarely 1 fertile); leaves often acute or apiculate, their apices usually deflexed; underleaves large, frequently cordate or subcordate, divided rarely as much as ⅛, *often* entire or retuse. Cells usually with minute trigones and few or no intermediate thickenings. Perianths 5-carinate, the carinae unmodified, the beak distinct. ..... *Hygrolejeunea*

Using the criteria in the preceding key, the following new species of Lejeuneaceae must be referred to the genus *Hygrolejeunea* and may be described as follows:

Hygrolejeunea alaskana Schuster & Steere, sp. nov. Plantae gregariae, pallide viridis, laxi, flaccidi, 0.9–1.0 (–1.25) mm. crassi. Cellulae caulis inferiores corticales 40–48 μ latae. Folia flaccida, delicata, lobis ovate falcata, apice deflexa, paulum geniculatis, alte ad carinae apicem emarginatis. Lobuli magni, anguste ovati, inflati, oblique positi; carinae arcuata convexa. Folia inferiora magna, superficialiter 0.5–0.7 parviora quam lobuli, ovata, basi rotunde cordata, bifida, sinu occulto angustissimo.

Autoecia. Gynoecia perlonga in caulibus primariis posita, innovationibus plerumque duabus. Perianthia maturitate stipitata, clavata, 5-carinata, carinis, laevibus.—Specimina typica a W. C. Steere lecta, prope vicinitatem "Driftwood Camp" dictum in montibus "De Long Mountains, Brooks Range" denominatis, Alaska.

Plants forming loose, whitish-green to pale yellowish-green patches in pure tufts or creeping over and among other bryophytes. Shoots lax and rather flaccid, 900–1050 (1225) μ wide, × 1–2 cm. long, rather sparsely, irregularly monopodially branched; stems 100–120 μ in diameter, with a pellucid, large-celled hyalodermis (ventral cortical cells 40–48 μ wide × 50–75 μ long), surrounding leptodermous medullary cells of much smaller diameter. Leaves moderately imbricate, flaccid and delicate, the dorsal lobe *ca.* (620) 700–800 μ long (from keel) × (450) 480–650 (600) μ wide (at notch between keel and free dorsal lobe), ovate but somewhat geniculate-falcate, the basal portion (proximal of apex of lobule) suberect, spreading at an angle of 40–45°, the free distal portion suddenly widely spreading, at an angle of 90–100°; dorsal lobe *very strongly convex, the broadly rounded apex broadly deflexed;* antical base subcordate, ampliate and strongly arched, *extending across and some distance beyond the stem* (in

antical aspect the stem quite hidden by the shingled leaves); postical margin evenly arched, from the *sharp incision at juncture with keel*, to the broadly rounded apex. *Lobule large, suberect* (at an angle of 38–45° with stem), strongly inflated, uniformly well developed, *narrowly ovate* (225) 260–325 μ long (to apical tooth) × (130) 140–160 μ wide, its length between 0.35–0.45

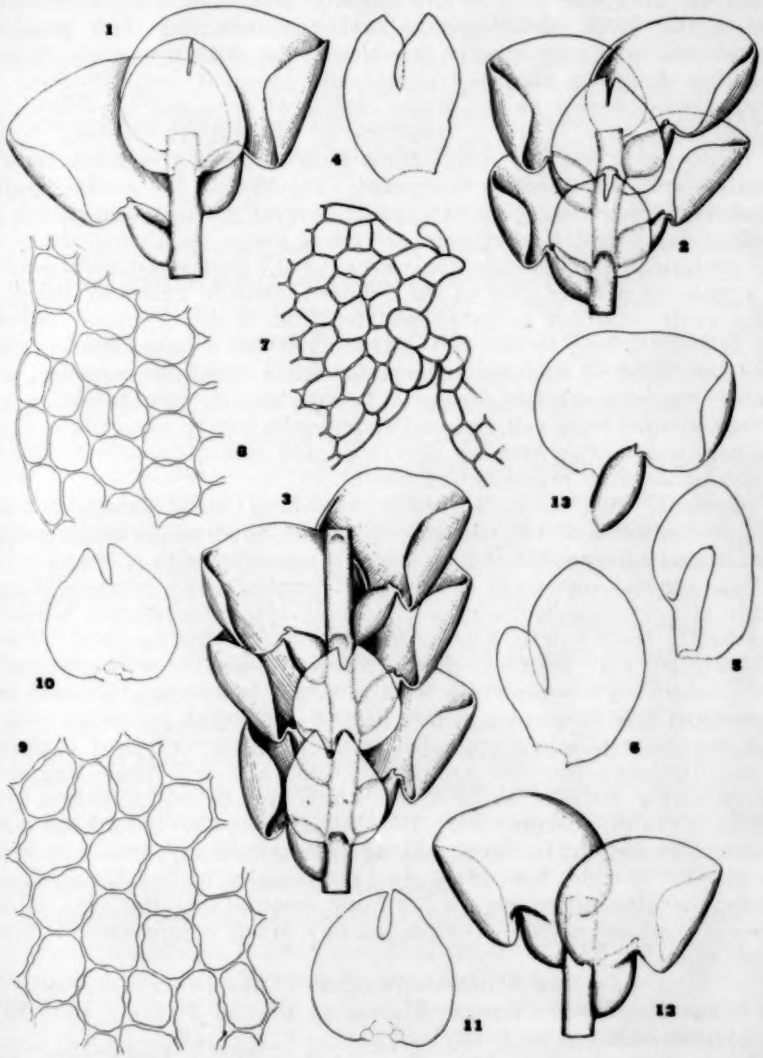

FIG. 1. *Hygrolejeunea alaskana* Schuster & Steere. 1–3. Sectors of shoots, postical aspect (×35); 4. Bracteole (×35); 5–6. Bracts (×35); 7. Apex of lobule and adjacent postical leaf-margin (×195); 8. Marginal cells (×300); 9. Median cells (×300); 10–11. Underleaves (×35); 12. Shoot-sector, antical aspect (×35); 13. Leaf, postical aspect (×35). (Drawn from type specimen).

that of dorsal lobe; free margin of lobule strongly involute, to and including the obtuse 1-celled apical tooth, the *mouth of the lobule strongly narrowed and constricted*, opening by a very slight, circular opening; *keel strongly convex*, the distal half at an acute (25–55°) angle with the posterior margin of lobe, *the consequent notch deep, sharp and salient;* hyaline papilla clavate, inserted on proximal base of the slightly elongated and barely curved, obtuse, apical tooth. Cells large, *strongly convex* (but free margins of marginal cells only very weakly crenulate), the walls somewhat thickened, with rather small but discrete trigones, and frequent, but ill-defined intermediate thickenings (the *cell-walls somewhat irregular and undulate*); marginal cells 16–21 (24) μ, quadrate to tangentially rectangulate, the walls thinner and less collenchymatous than in median cells; median cells subisodiametric, polygonal to hexagonal, (18) 19–22 (25) $\mu \times$ 24–30 μ long; ocelli absent; oil-bodies ($\pm$ disintegrated) several per cell, segmented, small (less than 8 μ long). *Underleaves extremely large*, on all but the weakest shoots *contiguous to imbricate*, their area 0.5–0.7 that of adjacent leaf lobes (but appearing greater, due to the deflexed-involute apices of the latter), broadly ovate-orbicular to ovate-cordate, from a minimum of 370–400 μ wide $\times$ 380–420 μ long to 465–530 μ wide $\times$ 490–550 μ long; apex narrowed, *bilobed for 0.25–0.35 its length*, the sinus either closed or narrowly, deeply V-shaped, the lobes erect to $\pm$ connivent; base broadly rounded on each side to a large, swollen basal cell, *in some underleaves barely subcordate;* rhizoid-initial field small, often scarcely developed, the rhizoids sparse or absent on most shoots. Asexual reproduction absent.

Autoecious.[5] Androecia intercalary on long, leafy shoots, compactly spicate, 605 μ wide, of 3–5 (occasionally more?) pairs of closely imbricate bracts; bracts subequally bilobed, strongly concave, with suberect or erect lobes, the ventral somewhat shorter and smaller than antical, obtuse or subacute at apex; bracteoles present throughout, suborbicular, bilobed for *ca.* one-fourth their length, similar to underleaves (but smaller). Gynoecia on elongate leafy (? leading) shoots, *almost invariably with 2 equal subfloral innovations*, exceptionally with a single innovation. Female bracts and bracteole free from each other; bracts (somewhat juvenile) with lobe oblong-obovate to elliptical, rounded at apex, entire-margined (except for faint crenulations), 650–680 μ long $\times$ 360–450 μ wide; lobule lingulate to narrowly oblong, rounded at the apex (which may be retuse, with a hyaline papilla in the faint emargination), 100–180 μ wide $\times$ 430–460 μ long, free for *ca.* one-half its length. Bracteole oblong-ovate to oblong-obovate, 575–600 μ long $\times$ 430–450 μ wide, bilobed $\frac{1}{3}$–$\frac{2}{5}$ (occasionally to $\frac{1}{2}$) the lobes erect, subacute; margins entire, except for faint crenulations. Perianth in maturity longly stipitate, clavate-obovoid, not or scarcely compressed, 5-carinate, the keels smooth.

Type: Driftwood Camp, near headwaters of the Utukok R., north slope of De Long Mts., Brooks Range, Alaska, *ca.* 68° 53′ N. Lat., 161° 10′ W. Long. (*Steere* 16900, Aug. 4–16, 1951).

Cotypes: Same locality data (*Steere* 16782, 16856, 16895, Aug. 11, 14 and 15, 1951); Titaluk Camp, in seepage area, on north-facing hillside,

[5] Because of the fragility of the material, autoecious inflorescences could not always be demonstrated. In two stems or plants, however, the connection between androecial and gynoecial shoots were clearly demonstrable.

hills north of Colville River, east of the Ikpikpuk River, approximately 68° 30′ N. Lat., 154° 30′ W. Long. (*Steere* 17037, Aug. 24, 1951).

This interesting hepatic was first found in the field in the vicinity of Driftwood Camp, near the confluence of Driftwood Creek with the Utukok River, some 130 miles south of Wainright, on the north slope of the De Long Mountains, the westernmost complex of the Brooks Range. It occurs in abundance in seepage channels or in springy natural drainage areas on steep, north-facing hillsides. At first it was overlooked because of its close resemblance in color and habitat (but not habit) to *Radula prolifera* Arn., an extremely common and abundant species in the foothills of the Arctic Slope of Alaska. However, *Radula prolifera*—as its name suggests—branches extensively and spreads through mosses and other plants under whose shelter it grows. *Hygrolejeunea alaskana*, on the other hand, grows usually in pure stands of long and relatively unbranched stems. Its distribution in Alaska is apparently restricted as it was discovered in only two areas during the summer of 1951, and searched for carefully during the two following summers—but unsuccessfully. Even at Cape Lisburne, at the same latitude and some 130 miles west, which has many unusual and extremely interesting bryophytes, the *Hygrolejeunea* was not found.

The ecological association of *Hygrolejeunea alaskana* with a group of other hepatics of unusual interest and rarity in North America, as *Ascidiota blepharophylla* Mass., *Frullania jackii* Gottsche, *F.* cf. *tamarisci* (L.) Dum., *Radula prolifera* Arn., *Lepicolea fryei* Perss., *Scapania simmonsii* (Arn.) Bryhn & Kaal., *S. spitzbergensis* (Lindb.) K. Müll., *Mesoptychia sahlbergii* (Lindb. & Arn.) Evans, and still others, indicates beyond question its membership in a flora of unusual phytogeographical interest, without doubt a relict flora that has persisted in unglaciated areas north of the Arctic Circle since Tertiary times (Steere 1953). The discovery of the *Hygrolejeunea* near Titaluk Camp, nearly 200 miles east and north of Driftwood Camp, means that this species probably has a wider distribution in arctic Alaska than our present collections and careful searches indicate, even though it will almost certainly be found to be restricted to unglaciated areas along the northern foothills of the Brooks Range.

This isolated and discrete species is assigned to the genus *Hygrolejeunea* with some misgivings. It has superficial points of similarity with *Macrolejeunea* Spruce, *Cystolejeunea* Evans, *Taxilejeunea* (Spr.) Schiffn., and *Peltolejeunea* (Spr.) Schiffn. Indeed, it demonstrates the great need for a sharper generic definition of these taxa. The similarities with these genera all revolve about the general facies of the plant: the large, orbicular or suborbicular underleaves, shortly bilobed at the apex; the inflated lobule, with a proximal hyaline papilla; the pellucid cells of relatively large size; the absence of ocelli; the robust size, combined with a considerable delicacy; and

the obclavate to obpyriform perianth with unarmed keels. These features, to a greater or lesser extent, are common to all the genera in question. *Cystolejeunea* can be eliminated from further consideration by three features that separate it from *H. alaskana:* the strongly collenchymatous cells, with bulging trigones; the non-carinate perianth; and the peculiar form of the lobules (see Evans 1906). The rounded apices of the leaves, the frequent presence of two subfloral innovations, and the scattered, rather than sympodial inflorescences separate *H. alaskana* from any species of *Taxilejeunea* known to the writers. The uniformly and rather deeply bilobed underleaves, inserted by an almost transverse line, and the stem anatomy separate *H. alaskana* from *Peltolejeunea.*

We should consider the relationship of *Macrolejeunea* to *Hygrolejeunea.* The former was characterized by Evans (1906, p. 17) as "a genus of somewhat doubtful validity. . . . In this . . . the female branch is simple, and the perianth is destitute of both beak and keels, characters which it shares with *Cystolejeunea.* It differs, however, in its general habit, in its pointed leaves and in its delicate texture, agreeing in all these respects with . . . *Taxilejeunea* and *Hygrolejeunea.*" The perianth form, the pointed leaves and the apparent constant lack of innovations below the ♀ branch all separate *Macrolejeunea* from the plant here described as *Hygrolejeunea alaskana.*

From the preceding it is evident that good reasons can be adduced for presuming an absence of generic identity of this plant with the preceding four genera. Similarly good reasons cannot be given for removing the plant from *Hygrolejeunea.* The latter genus is characterized by Vanden Berghen (1948, p. 27) as "plutôt delicates, pâles . . . cellules foliaires grandes, hyalines, à membranes minces . . . Amphigastres très grands, circulaires ou reniformes, souvent cordés, brièvement bifides. . . . Inflorescence femelle à l'extrémité d'un rameau pourvu d'une innovation sous-florale. . . . Perianthe pyriforme, plus ou moins comprime, présentant cinq carines lisses. . . ." This diagnosis of the genus agrees with the characters of the plant here described, with the sole exception that the ♀ inflorescence usually possesses 2 subfloral innovations. This scarcely can be considered as sufficient to exclude the species from the genus.

Stephani (1914) lists 101 species of *Hygrolejeunea,* and adds 5 more species a decade later (Stephani 1923). These species are exclusively tropical and subtropical, a few ranging northward in the New World to Cuba (*H cubensis* St., *H. sacculata* St., *H. wrightii* St.) and to Mexico (*H. aspera* St., *H. cerina* (L. and L.) Schiffn., *H. flavicans* (G.) St.). The occurrence of the genus in northern Alaska is phytogeographically most interesting in demonstrating the relict occurrence, in that area, of species whose affinity is with tropical taxa (Steere 1953).

Hygrolejeunea herzogii is characterized by the following combination

of features: (1) the delicate, strongly-deflexed lobes of the leaves; (2) the 5-carinate, at maturity exceptionally longly stipitate perianth; (3) the broadly rounded apices of the leaves; (4) the extremely strongly inflated lobules, suberect to obliquely spreading, the convex keel at a sharp, acute angle with the widely spreading posterior margin of the leaf; (5) the very large underleaves, rounded to subcordate at the base. These features serve to separate it from all other North American Lejeuneaceae. The presence of autoecious inflorescences sets the species apart from all other potentially allied species of *Hygrolejeunea*. According to the classification of Stephani (1914), *H. herzogii* would go into the "Fissistipulae obtusifoliae." Among the New World representatives of this group a few (*H. bahiensis* St., *H. compacta* (G.) St., *H. corynantha* Spr., *H. deplanata* (N.) St., and *H. tonduzana* St.) are autoecious. Some of these, such as "*H.*" *deplanata* and *bahiensis* bear a single subfloral innovation, and do not appear to belong in *Hygrolejeunea*. A relationship with one of the three remaining species cited remains a possibility, although *H. tonduzana* differs in the broader-than-long underleaves, *H. corynantha* in the very small, spicate androecium and smaller underleaves, and *H. compacta*, in the smaller cells, brownish color, and acute lobes of the perichaetial bracts.

A part of the original material of *H. alaskana* was sent to the eminent Japanese hepaticologist, Dr. S. Hattori, who suggested a similarity to *Lejeunea compacta* St. (*Lejeunea mayebarae* Hatt.), and kindly supplied material for comparison. Although some similarity exists between the two, the latter species differs very considerably in the uniformly cladogynous inflorescences (which are subtended by either a single innovation, or, more usually, without innovation), the much more pachydermous cells, with a delicately verruculose cuticle; the broader, usually broader than long, underleaves, whose sinuses are more open and whose lobes are more divergent; the much less prominently deflexed leaf-apices. The cladogynous inflorescences suggest that this species belongs in *Lejeunea* s. str., rather than in *Hygrolejeunea*. Consequently, in spite of the strong superficial similarities, no close phylogenetic relationship can be assumed. Dr. Th. Herzog, whose knowledge of the tropical Lejeuneae is without parallel, examined a portion of the type of *H. alaskana* He also noted the "*meist achselständige* Stellung des Perianths (besser Involucrum) . . ." and therefore considered the plant, as do the authors, more correctly placed in *Hygrolejeunea* than in *Taxilejeunea*.

In all probability a close affinity to the neotropical species mentioned above does not occur. Rather, *H. alaskana* is probably allied to the "old" flora of the Himalayas and Nepal. *Hygrolejeunea princeps* Steph., from Nepal, and *H. obscura* (Mitt.) Steph., from the Himalayas, are presumably more closely allied to our plant. The latter species differs considerably from

H. alaskana in (a) the cells devoid of trigones; (b) the lobuli very small, often obsolete, only to one-fifth the length of the lobe; (c) the keel of the lobuli nearly straight, not at a sharp angle with free dorsal lobe.

However, *H. princeps* appears to be a close ally of our plant. It agrees with it in the strongly convex leaves, with the keel of the lobule obliquely ascending, in the rounded antical base of the lobe, and in the majority of other characters. However, according to Stephani's diagnosis, *H. princeps* differs from *H. alaskana* in the following features: (a) plants brownish, rather than yellowish-white; (b) leaves contiguous rather than closely imbricate and obliquely rather than widely spreading; (c) lobe apices obtuse, rather than broadly rounded; (d) keel of lobule nearly straight, rather than highly arched; (e) the incision between keel and free dorsal margin of lobe rectangulate, rather than sharply acute; (f) underleaves six times as wide as stem, rather than 3.5–4.5 × as wide as stem.

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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 85, No. 3, pp. 197–200 MAY, 1958

VEGETATION AS A MEASURE INDICATOR OF AIR POLLUTION[1] PART I. THE PINE (PINUS-TAEDA)

F. W. BIEBERDORF, C. L. SHREWSBURY, H. C. MCKEE AND L. H. KROUGH

In connection with a study of air pollution in Harris County, Texas, an opportunity was afforded to study the Pine Tree *Pinus taeda* as an indicator of the degree of air pollution. The vegetative area studied comprised the whole of Harris County, Texas, with sampling carried out in accordance with a predesigned plan shown in the accompanying map.

Sampling was concentrated in areas of greatest industrial activity, which occurs along the Houston Ship Channel, where the greatest air pollution was suspected. In the outlying areas of the county, far removed from industrialization, fewer samples were taken.

Historical. Some of the earliest observations of vegetation damage resulting from air pollution were made by Camaron in 1874. Schroder and Schertel described the effects of smelter smoke on vegetation in 1844.

About 1900, the effect of illuminating gas upon green house plants aroused much interest. This was followed by a study of the lethal effects on plants of ethylene, acetylene, propylene, and carbon monoxide by Crocker, at Boyce Thompson Institute (1948). Since that time a great deal of research has been devoted to the study and it has become clear that some plants are better as indicators of air pollution than others.

Since one of the main air pollutants was found to be SO_2 the literature was examined particularly to determine its effects on various plants.

In the report of the Selby Smelter Commission, published in 1915, experiments showed that SO_2 vapors, under various conditions of humidity, temperature, and concentration of SO_2 gas, were injurious to barley. Trees in the area of the smoke zone of the smelter exhibited leaf damage similar to that caused by SO_2. This report describes and contains photographs of the type and character of leaf damage obtained by exposing barley to various concentrations of SO_2. The report describes the use of various plants as indicators of air pollution. The plants used were Eucalyptus, turnip, nasturtium, apple, pear, plum, grape, potato, rose and tomato. The symptons of SO_2 injury on these plants were photographed and described. The report concludes that in the case of SO_2, the plants used were excellent and sensitive guides to SO_2 concentration in the air.

In more recent work, Thornton and Setterstrom, of Boyce Thompson Institute, studied the effect of several gases, including sulfur dioxide, on

[1] This research was a part of the Greater Harris County Air Pollution Study which was supported by the Houston Chamber of Commerce.

green plants. The materials were tomatoes, buckwheat and tobacco. They found SO_2, as well as other gases, brought about injury as a result of a change in pH and that injury was greater when the plants were held in sunlight.

Katy and McCallum, in an article published by the National Research Council of Canada (1939), report sulfur analysis on one, two, and three year old yellow pine and Douglas Fir needles.

Specimens were selected at various distances from the source of sulfur dioxide emission. They found that conifers accumulate sulfur compounds from the atmosphere rapidly in young leaves and more gradually in older

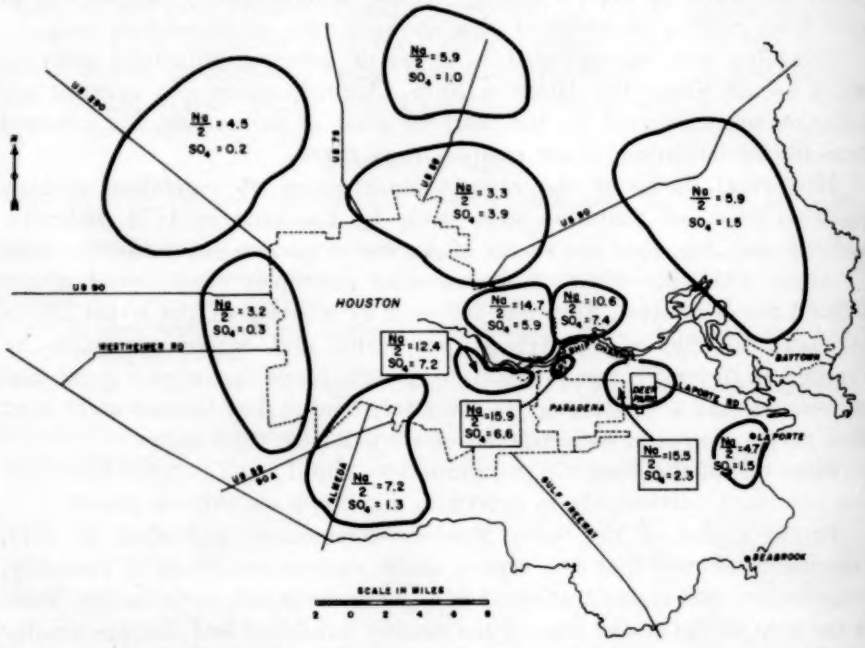

FIG. 1. Results of chemical analysis of pine needles (micro-moles per gram).

leaves. They found fir to be a more sensitive indicator of sulfur dioxide occurrence than yellow pine. They also found that plants may accumulate sulfur compounds in the leaves in amounts three or four times greater than normal without injury or retardation in growth and that within limits there is no relation between sulfur content and injury.

Experimental procedure. Since conifers apparently are good indicators of the presence of sulfur dioxide in the atmosphere an extensive sampling of pine *Pinus taeda* was made in this study; a total of 94 first and second year needles were analyzed for sulfur. Figure 1 shows the location of the sampling points. The leaves or needles were separated into one and two

year needles and analyzed separately but no differences in sulfur content were found. The needles were collected during the month of January, 1957, at a time when the trees were in a low state of activity. The leaves were analyzed for sulfate and sodium. Sulfate concentrations are expressed in micro-moles per gram of dry sample. Sodium concentrations are expressed as micro-moles of sodium per gram, divided by 2. This factor was used to permit a direct comparison on the basis of sodium sulfate (Na_2SO_4).

Results and conclusions. Examination of the trees and leaves of the pine at the various sampling areas showed no visible injury. This was true regardless of the sulfur concentration in the leaves.

The data from chemical analysis are reported in Table I.

TABLE I. *Sulfur content of pine leaves as an indicator of air pollution. Figures are micro-moles per gram (dry).*

Direction From Ship Channel	Distance in Miles From Ship Channel	Sulfate	Sodium 2
South	1–2	6.9	14.1
North	1–3	7.4	10.6
Northwest	1–4	5.9	14.7
Southeast	2–5	2.3	15.5
Northwest	3–10	3.9	3.3
Southeast	6–10	1.5	4.7
Southwest	5–15	1.3	7.2
West	10–15	0.3	3.2
Northeast	10–15	1.5	5.9
Northwest	12–20	1.0	5.9
West & North	10–25	0.2	4.5

A study of the sulfate concentrations in different areas studied gives a good indication of the pollution patterns of the area, regardless of the agent or agents responsible.

A comparison of the values obtained far removed from the industrial center gives a good indication of the distance that pollutants can be transported before being dispersed.

Examination of results from the various areas indicates that the sulfate values cannot be attributed to sodium sulfate alone. For example, the samples collected in one area contained one of the highest averages of sodium concentration, yet the figure for sulfate was much lower than many other areas. Moreover, many areas showed a considerable excess of sodium over sulfate indicating that pine needles are capable of accumulating sodium salts.

The data in the tables show that there is a definite correlation between the sulfate content of the pine needles and the distance of the sample from the industrial area.

This indicates that the pine can be used with confidence as a measure of the degree of air pollution in a given area.

The analysis of the pine needles for sulfate content gives an overall picture on pollution for the average period of the life of the needles.

In this study it was only possible to collect one and two year needles, because the three year needles had already been shed. Where it is possible to collect three year needles additional data could be obtained for air pollution conditions over a longer period.

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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 85, NO. 3, PP. 201–214 MAY, 1958

DIFFERENTIAL CHROMOSOME SEGMENTS IN EIGHT SPECIES OF TRILLIUM

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Exposure to cold treatment for a sufficient period of time produces regions of differential reactivity along the length of metaphase and anaphase chromosomes in *Trillium*. These segments appear to be understained and of reduced diameter. According to Darlington and La Cour (1938) each chromosome has a characteristic pattern of differential segments, although the size of the segment may be subject to some variation. They further suggest that differential reactivity may be used as a genetic and taxonomic indicator, a criterion of variation between species. Studies by Wilson and Boothroyd (1940 and 1944) and Bailey (1949) show that the pattern of differential segments produced as a result of cold treatment is not the same in *T. erectum* L. collected in Canada and *T. erectum* L. collected in Tennessee. Bailey (1954b) has further shown that the pattern of differential segments is not constant in *T. grandiflorum* (Michx.) Salisb. and *T. erectum* L. when these species are collected from widely separated areas. Bailey (1954b) suggests, however, that certain regions of differential reactivity appear to be constant as to position within these species and that they can probably be separated from each other on the basis of their patterns of differential segments. Fourteen such segments were shown to appear regularly in *T. erectum* L., whereas eighteen segments appeared to be constant in *T. grandiflorum* (Michx.) Salisb.

Attention has also been given to the length of individual chromosomes after exposure to low temperatures as well as the length of differential segments. Regarding the total length of chromosomes, Darlington and La Cour (1940) report that the chromosomes remain their usual length after cold treatment. Wilson and Boothroyd (1944) and Bailey (1949) observed greater than normal mitotic contraction in the chromosomes of *T. erectum* L. after cold treatment. Salord (1949), however, has demonstrated that excessive contraction following treatment does not take place in the chromosomes of *T. grandiflorum* (Michx.) Salisb. and *T. luteum* Harbison. Bailey (1954c) concludes that increase or decrease in total length of the diploid chromosome set as a result of cold treatment is not constant within a species, some collection of the same species showing an increase in total length, others a decrease.

The length of the differential segments themselves also appear to vary within a species. Darlington and La Cour (1940) conclude that the size

of the segment may be subject to some variation, and Bailey (1954c) also concluded that these segments are not constant as to length.

Bailey (1954c) has suggested that although the entire pattern of differential segments may vary within a species, there may be certain differential segments along the length of particular chromosomes which are characteristic for a given species. It is the purpose of this study to report the pattern of differential segments found along the length of metaphase and anaphase chromosomes in eight species of *Trillium*. Further studies may result in establishing a pattern of regularly appearing differential segments which can be used as a taxonomic indicator.

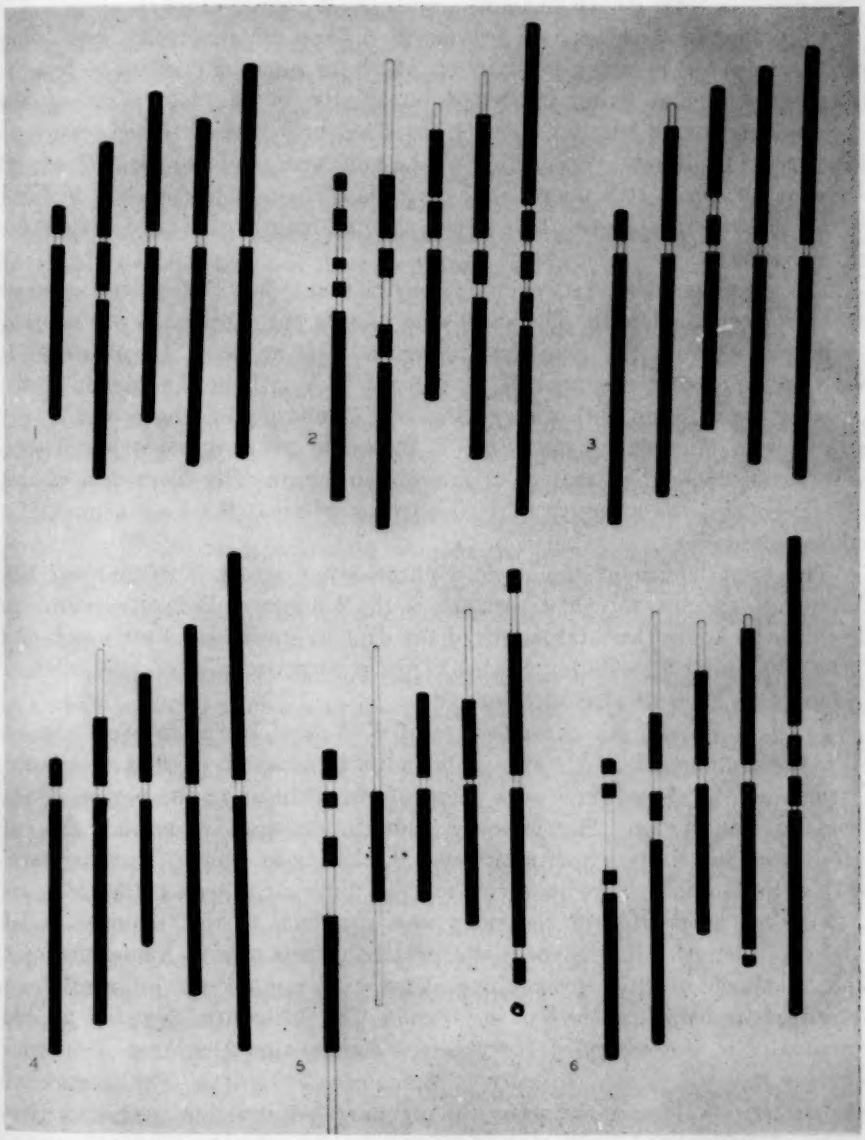
Materials and methods. Eight different species of *Trillium* were used in this study. Rhizomes of the several species were collected from various parts of the country and identifications were made by those individuals furnishing the rhizomes. Herbarium specimens were furnished in several instances and identifications were checked from these. Rhizomes of *T. ovatum* Pursh and *T. petiolatum* Pursh were collected near Pullman, Washington by Dr. Arthur J. Cronquist of the New York Botanical Gardens, formerly of the State College of Washington, Pullman, Washington. Rhizomes of *T. ludovicianum* Harbison were collected by Dr. Erdman West near Gainsville, Florida, and *T. Vaseyi* Harbison and *T. Catesbaei* Ell. from Highlands, North Carolina by Mr. Henry Wright. *T. flexipes* Raf. was collected by Dr. R. F. Thorne of the University of Iowa and Dr. Ray C. Friesner of Butler University. Rhizomes of *T. cernuum* L. were collected by the author near Alexander City, Alabama, and others were furnished by Dr. Arthur L. Cohen of Oglethorpe University. Rhizomes of *T. nivale* Riddell were furnished by Dr. Frank T. McFarland of the University of Kentucky, Dr. T. G. Yuncker of DePauw University, Dr. A. T. Guard of Purdue University, and Dr. R. F. Thorne of the University of Iowa.

All rhizomes used in this study were potted in peat moss and fresh growing root tips were used as a source of material. Cold treatment was provided with a commerical electric refrigerator which maintained a fairly constant temperature at 3° C. All tips used were exposed to this temperature for 96 hours.

All material for study was prepared using the Feulgen technique together with a squash method (Woodard 1948). All measurements were made on metaphase chromosomes using an ocular micrometer.

Observations and discussion. *Trillium Catesbaei.* The regularly recurring pattern of differential segments produced as a result of exposure to 3° C. for 96 hours in *T. Catesbaei* Ell., collected from near Highlands, North Carolina is shown in figure 1. Chromosomes A, C, D, and E show no regions of differential reactivity aside from their regions of the primary constric-

tion, whereas chromosome B shows one differential segment in addition to that of the primary constriction. This is a sub-median segment located in the long arm of the chromosome.



FIGS. 1–6. Diagrammatic analysis of chromosome morphology following cold treatment in several species of *Trillium*. FIG. 1, *T. Catesbaei* Ell. FIG. 2, *T. Vaseyi* Herbison. FIG. 3, *T. cernuum* L. FIG. 4, *T. ludovicianum* Harbison. FIG. 5, *T. nivale* Riddell. FIG. 6, *T. flexipes* Raf.

The primary constriction which acts as a differentially reactive segment in chromosome A measures .8 microns. The short arm of this chromosome measures 1.6 microns whereas the length of the long arm is 13.4 microns. This makes a total length for chromosome A of 15.8 microns.

Chromosome B shows two regions of differential reactivity, one being the region of the primary constriction which measures .4 microns in length; the second region being located sub-medially in the long arm of the chromosome with a length of .6 microns. The short arm of this chromosome measures 5.1 microns. The portion of the long arm distal to the differential segment measures 10.3 microns whereas the proximal portion of this arm is 2.5 microns in length. This gives a total length of 18.9 microns for chromosome B.

Chromosome C shows its primary constriction to be a differential segment of 1.1 microns in length. The short arm of this chromosome is 8.0 microns in length whereas the long arm measures 10.0 microns. Chromosome D shows a differential segment of .8 microns in length in the region of the primary constriction with a short arm of 6.7 microns in length and a long arm of 12.8 microns. Chromosome E shows its primary constriction as a differential segment measuring .7 microns in length. The short arm of this chromosome shows a length of 10.0 microns whereas the long arm is 12.3 microns long.

The total length of the diploid chromosome set in *T. Catesbaei* Ell. following exposure to cold treatment is 194.2 microns. Before exposure to 3° C. for 96 hours the total length of the diploid chromosome set was found to be 206.6 microns (Bailey 1954a). This gives a decrease of 12.4 microns in length as a result of cold treatment.

Trillium Vaseyi. All chromosomes of *T. Vaseyi* Harbison show regions of reduced diameter and staining capacity as a result of cold treatment. Chromosome A shows three such segments in addition to the region of the primary constriction. The primary constriction appears as a region of differential reactivity which measures 1.9 microns in length. The short arm of this chromosome shows an almost median differential segment 1.2 microns in length. The portion of the short arm proximal to this segment is 1.1 microns in length whereas the distal portion of this arm is .8 microns long. The long arm of this chromosome shows two regions of differential reactivity, both being sub-median in position. The differential segment located proximally to the region of the primary constriction measures .8 microns whereas the one located distally is .9 microns in length. The portion of chromosome A located between the primary constriction and the more proximal differential segment is .6 microns in length; the portion between the two differential segments measures .8 microns in length; the portion distal to the latter segment is 11.1 microns in length. This makes a total length of 19.2 microns for chromosome A.

Chromosome B shows four differential segments, one located terminally in the short arm, two appearing in the long arm, and one being located in the region of the primary constriction. The primary constriction which appears as a differential segment is .7 microns in length. The terminal segment located in the short arm is 6.6 microns in length. That portion of the short arm located between the primary constriction and the terminal segment is 3.6 microns in length. The long arm of chromosome B shows two differential segments, the more proximal one measuring 2.8 microns in length and the more distal one showing a length of .4 microns; the portion of this chromosome arm located between the region of the primary constriction and the more proximal differential segment is 1.8 microns long; that portion between the two differential segments being 1.8 microns, whereas the more distal portion of the long arm shows a length of 9.9 microns. This gives a total length of 27.6 microns for chromosome B.

Chromosome C in this species of *Trillium* shows a total length of 17.2 microns with two differential segments being located in the short arm. These segments are in addition to the region of the primary constriction which shows a length of .8 microns. The terminal differential segment measures 1.6 microns whereas the sub-median segment shows a length of .4 microns. The portion of the chromosome arm proximal to the terminal segment is 3.8 microns in length, whereas the portion proximal to the sub-median segment is 1.8 microns long. The long arm of this chromosome is 8.8 microns in length, making the total length for the chromosome of 17.2 microns.

Chromosome D of *T. Vaseyi* Harbison shows a differential segment in each of its arms in addition to the region of the primary constriction. The short arm of this chromosome shows a terminal differential segment of 2.5 microns in length, whereas the differential segment in the long arm is a sub-median one measuring .8 microns in length. The differential segment at the point of the primary constriction is .8 microns in length. The non-differential portion of the short arm of chromosome D is 7.2 microns in length. That portion of the long arm between the region of the primary constriction and the sub-median differential segment is 1.9 microns in length. That portion of the long arm located distally to the sub-median segment measures 9.5 microns. These figures give a total length of 22.7 microns for chromosome D.

Chromosome E shows one sub-median differential segment in its short arm which measures .8 microns in length and two sub-median segments in the long arm which measure .8 and .4 microns respectively. In addition to these three segments, the region of the primary constriction appears as a differential segment with a length of .8 microns. The portion of the short arm distal to the differential segment measures 10.6 microns in length. The portion of the long arm between the region of the primary constriction and

the nearest sub-median segment is 1.6 microns in length; the portion between the two differential segments is also 1.6 microns in length, whereas the more distal portion of this arm measures 11.1 microns in length. This brings the total length of chromosome E to 29.3 microns.

A diagramatic analysis of the chromosomes of *T. Vaseyi* Harbison showing the differential segments produced as a result of cold treatment can be seen in figure 2.

The total length of the diploid chromosome set of *T. Vaseyi* Harbison following cold treatment was found to be 232.0 microns. Bailey (1954a) found the total length, before cold treatment, to be 203.4 microns in the same collection of *Trillium Vaseyi* Harbison.

Trillium cernuum. Chromosomes A, D, and E of *T. cernuum* L. collected from near Alexander City, Alabama and Oglethorpe University in Georgia fail to show regions of differential reactivity following cold treatment except in the region of the primary constriction. This region which does appear as a differential segment is .8 microns in length in each of the chromosomes, that is chromosomes A, D, and E. The short arm of chromosome A is 1.6 microns in length whereas the long arm measures 15.9 microns, giving a total length for chromosome A of 18.3 microns. Chromosome D shows a total length of 22.5 microns, its short arm measuring 9.4 microns and the long arm showing a length of 12.3 microns. The total length of chromosome E is 25.4 microns with a short arm of 11.5 microns and a long arm which measures 13.1 microns.

Chromosome B of *T. cernuum* L. shows a terminal differential segment in its short arm in addition to the region of the primary constriction. This terminal segment measures 1.3 microns in length whereas the segment at the point of the primary constriction is .8 microns in length. The non-differential portion of the short arm is 6.8 microns in length. The long arm of this chromosome measures 14.3 microns, giving a total length for the chromosome B of 23.2 microns.

Chromosome C of this species shows a differential segment of .5 microns in length at the region of the primary constriction, plus an additional differential segment in the short arm. The segment in the short arm is a sub-median one which measures 1.2 microns in length. That portion of the short arm located between the two segments is 1.6 microns in length whereas the distal portion of this chromosome arm measures 6.3 microns. This long arm shows a length of 10.4 microns. This brings the total length of chromosome C to 20.0 microns.

Figure 3 shows the chromosomes of *T. cernuum* L. with their pattern of differential segments produced as a result of cold treatment.

Bailey (1954) found the total length of the diploid chromosome set of *T. cernuum* L. to be 223.0 microns before cold treatment. This study shows

a total length for this species to be 218.8 microns after exposure to 3° C. for 96 hours.

Trillium ludovicianum. Chromosomes A, C, D, and E. of *T. ludovicianum* Harbison show only one differential segment each, this segment being the region of the primary constriction. This differentially reactive segment in chromosomes A and D measures .8 microns in length, in chromosome C 1.1 microns, and in chromosome E .9 microns. Chromosome A shows a total length of 17.3 microns with a short arm of 1.6 microns in length and a long arm of 14.9 microns. The total length of chromosome C is 16.0 microns with a short arm length of 6.3 microns and a long arm length of 8.6 microns. The short arm of chromosome D measures 9.3 microns, whereas the long arm of this chromosome shows a length of 11.6 microns. This gives a total of 21.7 microns for chromosome D. The total length of chromosome E is 29.2 microns. The short arm of this chromosome is 13.3 microns long, the long arm being 15.0 microns in length.

Chromosome B of *T. ludovicianum* Harbison shows a terminal differential segment of 4.1 microns in length in addition to the differential segment at the point of the primary constriction. This segment is .8 microns in length. The non-differential portion of the short arm is 4.0 microns in length, whereas the long arm is 13.3 microns in length. This gives a total length of 22.2 microns for chromosome B.

Figure 4 shows the pattern of differential segments resulting from cold treatment in *T. ludovicianum* Harbison.

The total length of the diploid chromosome complement in *T. ludovicianum* Harbison following cold treatment is 212.8 microns, whereas Bailey (1954a) demonstrated the total length of the chromosome set to be 223.0 microns before cold treatment.

Trillium nivale. Figure 5 shows the pattern of differential segments in *T. nivale* Riddell, produced as a result of cold treatment. Chromosome A of this species shows a differential segment in the region of the primary constriction which measures .8 microns. The long arm of this chromosome shows a terminal segment of 5.1 microns in length plus two additional intermediate segments of 1.8 and 3.0 microns in length respectively. That portion of chromosome A located between the segment which represents the primary constriction and the adjacent differential segment is .8 microns in length. The portion between the two intermediate segments in the long arm is 1.6 microns in length, whereas that portion between the terminal segment and the more distal intermediate segments shows a length of 7.9 microns. The short arm of chromosome A is 1.6 microns in length. This gives a total length of 22.6 microns for chromosome A.

Chromosome B of *T. nivale* Riddell. fails to show a distinct segment which can be associated as the region of the primary constriction, and it

also fails to show any other evidence of such region. The non-differential portion of this chromosome shows a total length of 6.5 microns. At each end of this portion there is a region of differential reactivity, one measuring 8.6 microns in length and the other showing a length of 6.2 microns. It is probable that the proximal portion of the differential segment which measures 8.6 microns in length represents the regions of the primary constriction. The total length of chromosome B is 21.3 microns.

The total length of chromosome C is 12.2 microns, the short arm measuring 4.8 microns and the long arm shows a length of 6.6 microns. The region of the primary constriction acts as a differential segment with a length of .8 microns.

Chromosome D shows the primary constriction to be a differential segment of .4 microns in length. The short arm of this chromosome shows a terminal differential segment which measures 5.4 microns in length. The non-differential portion of the short arm shows a length of 5.0 microns. The long arm of this chromosome measures 8.3 microns, giving a total length of 19.1 microns for chromosome D.

Chromosome E shows a sub-terminal differential segment in each arm in addition to the segment which represents the region of the primary constriction. The segment located at the primary constriction is .8 microns in length, the one located in the short arm is 2.6 microns in length, whereas the segment located in the long arm measures .7 microns. The portion of the short arm distal to the sub-terminal segment is 1.1 microns in length with that portion proximal to the segment measuring 8.3 microns. The proximal portion of the long arm shows a length of 9.4 microns, whereas the distal portion of this arm measures 1.4 microns. This gives a total length for chromosome E of 24.3 microns.

Bailey (1954a) demonstrated the total length of the diploid chromosome complement of *T. nivale* Riddell to be 176.4 microns in length before cold treatment. This study shows a total length of 222.2 microns for the diploid chromosome set following exposure to 3° C. for 96 hours.

Trillium flexipes. Figure 6 shows the pattern of differential segments produced as a result of cold treatment in specimens of *T. flexipes* Raf. collected near the University of Iowa and Butler University. Chromosome A of this species shows two sub-median differential segments in its long arm in addition to the segment which represents the region of the primary constriction. The more proximal segment of the long arm shows a length of 4.3 microns, whereas the more distal segment in this arm shows a length of .6 microns. The segment at the point of the primary constriction is .6 microns in length. The non-differential portion of the long arm located between the segment which represents the primary constriction and the more proximal segment is .8 microns in length. The portion between the

two differential segments is also .8 microns in length, whereas the distal non-differential portion of the long arm measures 9.8 microns. The short arm of chromosome A shows a length of .8 microns giving a total length of 17.7 microns for the chromosome.

The region of the primary constriction in chromosome B acts as a differential segment showing a length of 1.0 microns. The short arm of this chromosome shows a terminal segment with a length of 6.1 microns; the long arm show a sub-median segment which measures 1.2 microns. The non-differential portion of the short arm of chromosome B is 3.2 microns in length. The portion of the long arm located between the region of the primary constriction and the sub-median segment shows a length of 1.6 microns, whereas that portion of the long arm distal to the differential segment measures 12.2 microns. This gives a total length of 25.3 microns for chromosome B.

The short arm of chromosome C shows a terminal differential segment 3.1 microns in length. The non-differential portion of this arm has a length of 5.3 microns. The long arm of this chromosome has a length of 8.7 microns. The region of the primary constriction appears as a differential segment with a length of 1.3 microns. This brings the total length of chromosome C to 18.4 microns.

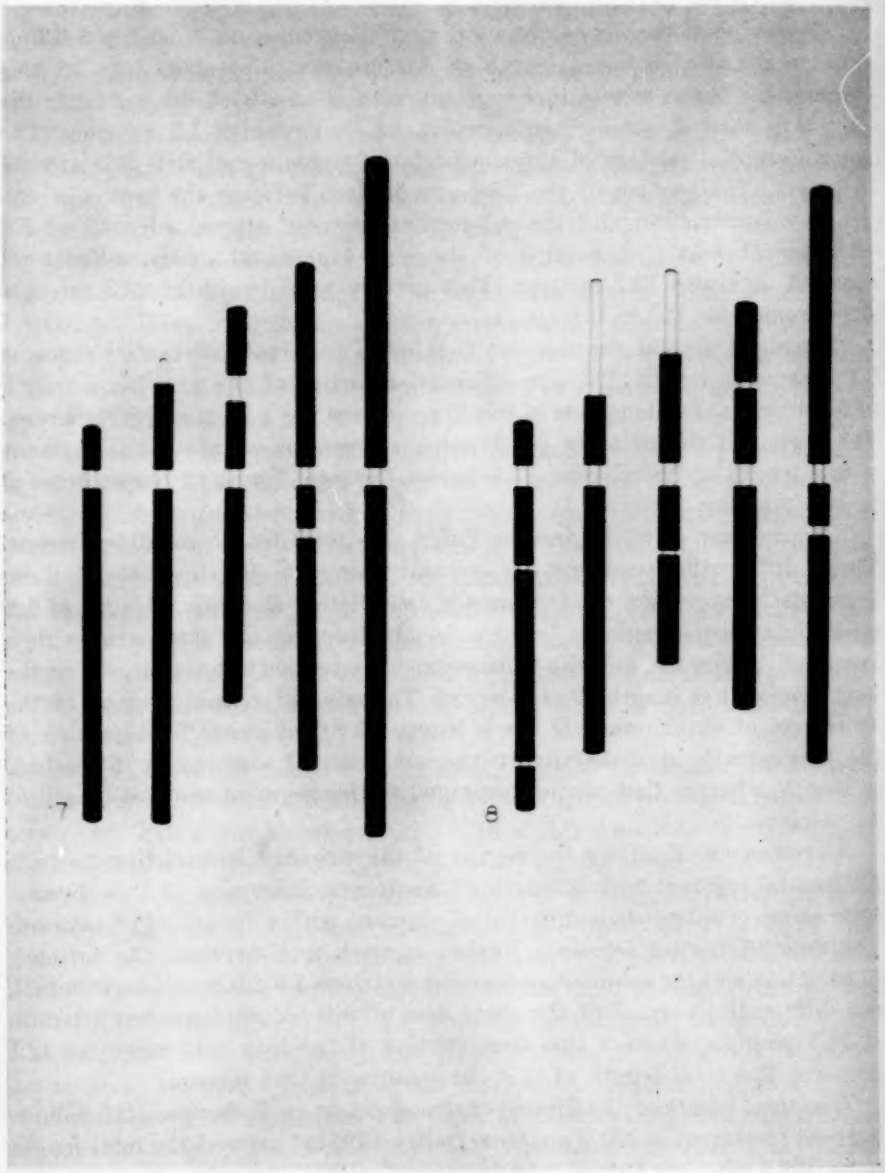
Chromosome D of *T. flexipes* Raf. has a total length of 20.9 microns. Three differential segments are evident along its length; one of these segments is the region of the primary constriction showing a length of 1.2 microns, a second segment being a terminal one in the short arm with a length of .8 microns, and the third segment is located sub-terminally in the long arm with a length of .4 microns. The non-differential portion of the short arm of chromosome D has a length of 8.0 microns. That portion of the long arm located distally to the sub-terminal segment is .6 microns in length, whereas that portion proximal to this segment shows a length of 9.9 mircons.

Chromosome E shows the region of the primary constriction to be a differential segment with a length of .8 microns. Each arm of this chromosome shows a sub-median differential segment with a length of .8 microns. The non-differential segments located in each arm between the primary constriction and the sub-median segment measures 1.6 microns. The terminal, non-differential portion of the short arm of this chromosome has a length of 11.1 microns, whereas this same portion of the long arm measures 12.1 microns. The total length of this chromosome is 28.8 microns.

The total length of the diploid chromosome set in *T. flexipes* Raf. following cold treatment is 222.2 microns. Bailey (1954a) showed the total length to be 196.4 microns before cold treatment within this same material.

Trillium petiolatum. Figure 7 shows the pattern of differential segments

produced as a result of cold treatment in *T. petiolatum* Pursh. Chromosomes A and E of this species show only one differential segment, that is the region of the primary constriction, each with a length of .8 microns. The



FIGS. 7–8. Diagrammatic analysis of chromosome morphology following cold treatment in two species of *Trillium*. FIG. 7, *T. petiolatum* Pursh. FIG. 8, *T. ovatum* Pursh.

short arm of chromosome A is 1.6 microns in length, whereas the long arm measures 12.9 microns. This makes the total length for Chromosome A of 15.3 microns. The long arm of chromosome E is 13.3 microns in length. The short arm of this chromosome shows a length of 11.7 microns, giving a total length for chromosome E of 25.8 microns.

The short arm of chromosome B of *T. petiolatum* Pursh shows a terminal differential segment of .8 microns in length in addition to the region of the primary constriction which also measures .8 microns in length. The non-differential portion of the short arm is 3.3 microns in length. The long arm of chromosome B measures 12.9 microns giving a total length of 17.7 microns for the chromosome.

Chromosome C shows its region of the primary constriction to be a differential segment with a length of .8 microns. The short arm of this chromosome shows a median differential segment 1.0 micron in length. The more proximal, non-differential portion of the short arm shows a length of 2.5 microns. The terminal portion of this arm also shows a length of 2.5 microns. The long arm of chromosome C shows a length of 8.3 microns, giving a total length for the chromosome of 15.1 microns.

Chromosome D of *T. petiolatum* Pursh has a total length of 19.4 microns following cold treatment. This chromosome shows its region of the primary constriction to be a differential segment of .8 microns in length. The long arm shows a sub-median segment which measures .4 microns in length. The proximal portion of this arm measures 1.6 microns whereas the distal portion shows a length of 8.9 microns. The short arm of chromosome D has a length of 7.7 microns.

Chromosome E shows its region of the primary constriction to be a differential segment with a length of .8 microns. The short arm has a length of 11.7 microns whereas the long arm is 13.3 microns in length. The total length for chromosome E is 25.8 microns.

The total length of the diploid chromosome set of *T. petiolatum* Pursh following cold treatment is 186.0 microns whereas the total length before cold treatment has been shown to be 214.0 microns (Bailey 1954c).

Trillium ovatum. Chromosome A of *T. ovatum* Pursh shows three regions of differential reactivity; one being the region of the primary constriction with a length of .8 microns, a second segment located sub-medially in the long arm with a length of .2 microns, and a third segment which is sub-terminal in the long arm also measuring .2 microns in length. The length of the short arm in this chromosome is 1.6 microns. The proximal, non-differential portion of the long arm has a length of 3.0 microns, the distal portion 1.6 microns, and the intermediate portion a length of 7.2 microns. This gives a total length of 14.6 microns for chromosome A.

Chromosome B shows a total length of 18.0 microns. The primary con-

TABLE 1. *Comparative data on chromosome morphology of some species of Trillium following cold treatment.*

Species	Chromosome A			Chromosome B			Chromosome C			Chromosome D			Chromosome E			TOTAL
	L.A.	S.A.	T.L.	L.A.	S.A.	T.L.	L.A.	S.A.	T.L.	L.A.	S.A.	T.L.	L.A.	S.A.	T.L.	
T. Catesbaei Ell.	13.4	1.6	15.8	13.4	5.1	18.9	10.0	8.0	19.1	12.8	6.7	20.3	12.3	10.0	23.0	194.2
T. Vaseyi Harbison	14.2	3.1	19.2	16.7	10.2	27.6	11.4	5.4	17.2	12.2	9.7	22.7	15.5	13.0	29.3	232.0
T. cernuum L.	15.9	1.6	18.3	14.3	8.1	23.2	10.4	9.1	20.0	12.3	9.4	22.5	13.1	11.5	25.4	218.8
T. ludovicianum Harbison	14.9	1.6	17.3	13.3	8.1	22.2	8.6	6.3	16.0	11.6	9.3	21.7	15.0	13.3	29.2	212.8
T. nivale Riddell	20.2	1.6	22.6	12.7	8.6	21.3	6.6	4.8	12.2	8.3	10.4	19.1	11.5	12.0	24.3	199.0
T. flexipes Raf.	16.3	.8	17.7	15.0	9.3	25.3	8.7	8.4	18.4	10.9	8.8	20.9	14.5	13.5	28.8	222.2
T. Petiolatum Pursh	12.9	1.6	15.3	12.9	4.1	17.7	8.3	6.0	15.1	10.9	7.7	19.4	13.3	11.7	25.8	186.0
T. ovatum Pursh	12.2	1.6	14.6	10.2	7.0	18.0	6.6	7.2	14.7	8.3	5.9	15.0	10.5	10.3	21.6	167.8

L.A.—Long arm; S.A.—Short arm; T.L.—Total length.

The length of the long arm plus the length of the short arm does not equal the total length given for individual chromosomes. This is because the length of the primary constriction which acts as a differential segment is not considered in the length of the long arm or short arm. For the length of these segments see descriptions of each species.

striction acts as a differential segment with a length of .8 microns. In addition, the short arm shows a terminal segment of 4.4 microns. The non-differential portion of the short arm is 2.6 microns. The long arm of this chromosome has a length of 10.2 microns.

Chromosome C shows three differential segments; a terminal segment in the short arm which measures 3.2 microns; a sub-median segment in the long arm with a length of .2 microns, and the region of the primary constriction with a length of .9 microns. The proximal, non-differential portion of the long arm has a length of 2.4 microns, whereas the distal portion of this arm measures 4.0 microns in length. The non-differential portion of the short arm shows a length of 4.0 microns, giving a total length for the chromosome of 14.7 microns.

Chromosome D has a total length of 15.0 microns following cold treatment. Two differential segments are evident in this chromosome, one being the region of the primary constriction with a length of .8 microns and the other a median segment of .3 microns in length located in the short arm. The distal as well as the proximal non-differential portion of the short arm shows a length of 2.8 microns. The long arm of this chromosome has a length of 8.3 microns.

Chromosome E show its primary constriction to be a differential segment with a length of .8 microns. One additional such segment appears in the long arm of this chromosome. This is a sub-median segment with a length of .4 microns. The proximal, non-differential portion of the long arm has a length of 1.6 microns, the distal portion a length of 8.5 microns. The short arm of chromosome E has a length of 10.3 microns, making a total length for the chromosome of 21.6 microns.

Figure 8 shows the pattern of differential segments produced as a result of cold treatment in *T. ovatum* Pursh.

The total length of the diploid chromosome set of this species following cold treatment is 167.8 microns. The total length before cold treatment has been shown to be 217.2 microns (Bailey 1954c).

Comparative data on the chromosome morphology of the eight species of *Trillium* included in this study is summarized in Table 1. Table 1 shows the total length of the chromosome complement after cold treatment as well as the length of individual chromosomes and the length of chromosome arms.

SUMMARY

1. The pattern of differential segments produced as a result of cold treatment in each of the eight species included in this study is different.

2. The increase or decrease in total length of the diploid chromosome set following cold treatment in each of the eight species is variable. *T. Catesbaei* Ell., *T. cernuum* L., *T. petiolatum* Pursh, and *T. ovatum* Pursh show a

decrease in the length of their diploid chromosome complements whereas *T. Vaseyi* Harbison, *T. ludovicianum* Harbison, *T. nivale* Riddell, and *T. flexipes* Raf. show an increase in length following cold treatment.

ALABAMA COLLEGE
MONTEVALLO, ALABAMA

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BULLETIN OF THE TORREY BOTANICAL CLUB

VOL. 85, NO. 3, PP. 215–219 MAY, 1958

TORREYA

Red Nuclei in Smilacina Fruits

Ripe red fruits of *Smilacina racemosa* (L.) Desf. were collected and examined October 6, 1957, near Ann Arbor, and it was found that these fruits derive their red color, not from chromoplasts or vacuolar pigments as might be expected, but from the nuclei in the cells of both pulp and epidermis. Other pigments seem to be entirely lacking. The nuclei are dull red in color and about 10 microns in diameter. It would be interesting to determine whether the fruits of other species of *Smilacina*, such as *S. stellata, sessilifolia,* and *amplexicaulis,* also have such nuclear pigmentation.

After the pigment was extracted by boiling, a positive Feulgen reaction with Schiff's reagent verified that these pigmented bodies are nuclei. The character of this nuclear pigment, however, is not known to the authors. Its solubility in water and in alcohol indicates the possibility of anthocyanin, but no color change was observed when tested with acetic acid, NH_4OH, and NaOH.

The problem of showing nuclei to elementary students has been traditional. These nuclei stand out conspicuously, and there are no plastids to mask them. These fruits make excellent demonstration material, for the cells can be prepared quite easily by squashing a ripe fruit on a slide and adding a drop of water and cover slip. Material can be gathered into December or can be kept for several months in a cold room at 40 degrees F., so it can be used both in the fall and spring semesters.—JOHN T. MICKEL and JAMES S. WILSON, Department of Botany, University of Michigan, Ann Arbor, Michigan.

PROCEEDINGS OF THE CLUB

November 19, 1957. The meeting of the Club at the Brooklyn Botanic Garden was called to order by President Rickett at 8:05 P.M. There were 29 members and guests present. The minutes of the October 19, 1957 meeting were accepted as corrected. Dr. Rickett announced that Mr. Joseph Monachino would serve as chairman of the Field Committee during 1958. Dr. A. J. Ullstrup and Dr. George E. Wheeler were elected to Active (Annual) Membership and Mrs. Ethel Luttinger to Associate Membership in the Club. The resignations of Mrs. C. T. Downey and N. C. Wycoff were accepted with regret.

President Rickett then introduced the speaker of the evening, Mr. Estil I. Green who spoke to the Club on, "Wild Orchids of New Jersey". Mr. Green gave a most interesting talk on the orchid flora of the state and illustrated it with superb colored slides of 48 of the 52 known species in New Jersey.

The meeting was adjourned at 9:07 P.M. after which refreshments were served by the members of the Garden Staff.

Respectfully submitted
Frank G. Lier
Recording Secretary

December 3, 1957. The meeting of the Club at Columbia University was called to order by President Rickett at 8:05 P.M. Forty three members and guests were present. The minutes of the November 19, 1957 meeting were accepted as read. Dr. Wray Bowden was elected to Active (Annual) Membership in the Club.

Dr. Nelson read a letter from the "Save the Dunes Council of Indiana" which requested that members of the Torrey Botanical Club write letters to the Governor of Indiana stating that a portion of the Indiana dunes be preserved in its natural state. Recent industrial development of the area threatens to wipe out the last remaining section of the dunes. Dr. Olive moved, seconded by Dr. Clum that the Secretary be empowered to write a letter in behalf of the Club. The motion was unanimously passed.

Dr. Rickett then introduced the speaker of the evening, Dr. Seymour H. Hutner

of the Haskins Laboratories who spoke on, "Chemical Ecology of Some Interesting Algae". Dr. Hutner's talk included material on the chemical nutritional requirements of several of the algal flagellates such as Ocromonas, Ceratium, and several others. The role of changing temperatures in its effect on the nutritional requirements of these algae was also presented.

After a question period the meeting was adjourned at 9:00 P.M.

Respectfully submitted
Frank G. Lier
Recording Secretary

January 7, 1958. The meeting of the Club at Columbia University was called to order by President Rickett at 8:05 P.M. Thirty-three members and guests were present. The minutes of the December 3, 1957 meeting were accepted as read. Dr. David Rogers of the New York Botanical Garden was elected to Active (Annual) Membership in the the Club. Mrs. Warren Kinney of New Vernon, New Jersey changed her membership from Associate to Sustaining Member.

Dr. Rickett then introduced the speaker of the evening, Dr. Ruth Thomas of Eastern New Mexico University who spoke to the Club on "Gametogenesis, Fertilization, and Development of the Proembryo in *Pinus virginiana*". Dr. Thomas illustrated her talk with excellent slides of photomicrographs showing the development of the male and female gametophytes, gametes, the process of fertilization, and the development of the proembryo.

Following a period of discussion the meeting was adjourned at 9:00 P.M. after which refreshments were served by the Club.

Respectfully submitted
Frank G. Lier
Recording Secretary

Book Reviews

Matériaux pour une monographie de *Trachelomonas* Ehrenberg, C., 1834, *Strombomonas* Deflandre, G., 1930, et *Euglena* Ehrenberg, C., 1832, genres d'Euglénacées. By W. Conrad and L. van Meel. Mem. No. 124, Institut Royal des Sciences Naturelles de Belgique, Rue Vautier 31, Brussels, Belgium. 176 pp., 19 pl., 1952. 4°.

This volume, issued September 30, 1952, presents posthumously the manuscript prepared by Walter Conrad for publication in 1930. Although long delayed in appearance, it proves to be a distinct contribution to the taxonomic knowledge of these genera of flagellates; and it merits attention of phycologists of this country. The senior author's original intent—to treat all European species—was extended greatly. Certain American, Asiatic and Australian entities are included. The work leans admittedly on the monograph by Deflandre (1926), and emphasis is given the Belgian species.

Included are 62 species of the genus *Trachelomonas*, 7 species of *Strombomonas*, and 41 species of *Euglena*. Latin descriptions of 9 new species, 10 new varieties, and 3 new forms are assembled at the end of the text. The new entities, together with their original localities, include *Trachelomonas kufferathi* var. *producta* Conrad, *nov. var.* (Australia), *T. rugulosa* var. *dangeardii* f. *inflexa* Conrad, *nov. f.* (Germany) and f. *torquata* Conrad, *nov. f.* (Germany), *T. rugulosa* var. *semi-ornata* Conrad, *nov. var.* (Germany) and var. *meandrina* Conrad, *nov. var.* (Germany), *T. umbilicophora* var. *deflandrei* Conrad, *nov. var.* (Venezuela), *T. denisii* var. *sphagnicola* Conrad, *nov. var.* (Belgium), *T. australica* var. *rectangularis* f. *invaginata* Conrad, *nov. f.* (Netherlands), *T. africana* var. *amphorula* Conrad, *nov. var.* (Belgium), *T. euchlora* var. *parvula* (Massart) Conrad, *nov. var.* [locality?], *T. reewijkiana* Conrad, *nov. sp.* (Netherlands), *T. lineata* (Massart) Conrad, *nov. sp.* (Belgium), *T. lychenensis* Conrad, *nov. sp.* (Germany), *Strombomonas acuminata* var. *deflandriana* Conrad, *nov. var.* [locality?], *S. ensifera* var. *pulcherrima* Conrad, *nov. var.* (Egypt), *Euglena rhynchophora* Conrad,

nov. sp. (Belgium), *E. inflata* Massart, *nov. sp.* (Belgium), *E. calva* Massart et Conrad *nov. sp.* (Belgium), *E. striato-punctata* Massart et Conrad, *nov. sp.* (Belgium), *E. ehrenbergii* var. *teres* Massart, *nov. var.* (Belgium), *E. variabilis* var. *piriformis* Massart et Conrad, *nov. var.* (Belgium), *E. proxima* var. *parvula* Kufferath et Conrad, *nov. var.* (Belgium) [lacks Latin description], *E. anura* Massart, *nov. sp.* (Belgium), *E. vittata* Massart, *nov. sp.* (Belgium).

The individual text for each taxon is brief and succinct; and aside from essentials such as name, author, and original bibliographic citation, documentation is minimal.

Van Meel has done an excellent piece of work in producing this manuscript. Of special usefulness or note are the Latin descriptions assembled under "*Specierum varietatumque diagnoses,*" the index to specific and varietal epithets for both accepted names and synonyms, the table and chart for distribution of Belgian taxa, and the extensive bibliography. The plates are useful, although it is unfortunate that Conrad's drawings of the new entities were not located nor included. For the most part of the text seems remarkably free of editorial errors, although on p. 153 "nov. sp." should read "nov. var." Nomenclatural documentation is not always obvious to the reviewer, as on p. 14 where *Trachelomonas americana* Lemm. is used rather than the earlier synonym *T. spinosa* Palmer.

The work is beautifully done in the quarto size and with the lavish lining, wide margins, and large print characteristic of the works of this Belgian institute. Ludo van Meel is to be congratulated for his dedicated service to a deceased associate.—R. D. Wood, Department of Botany, University of Rhode Island, Kingston, Rhode Island.

[**Note:** In the letter accompanying this review Professor Wood makes the following statement, "You will note that this article is a review of a monograph on Flagellates written in 1952. It has been my discovery that few Americans were acquainted with this volume." Ed.]

Handbuch der Pflanzenkrankheiten. Vol. II, Second part, 6th ed. Bakterielle Krankheiten. Paul Parey, Berlin & Hamburg. 567 pp. 1956.

The second part of this comprehensive work (see the Torreya section of the Bull. Torrey Bot. Club **82**: 402–403, 1955, for the review of the earlier volume on virus diseases), considered on merit alone, gives evidence of being a major contribution to the literature on plant diseases. The Editor, Dr. C. Stapp, and the publishers are to be congratulated for undertaking the long needed task of bringing together all bacterial diseases of plants. Thirty years have elapsed since the appearance of the previous edition. In this long span of time the international literature on the subject has increased greatly. The present volume contains full information on all newer and older work concerning bacterial diseases of plants, including diseases caused by actinomycetes.

The volume begins with a preface by Dr. Richter, who succeeded Dr. Otto Appel as Editor-in-Chief of the Sorauer Handbuch. The description of specific diseases follows the names of hosts arranged in the Engler and Prantl order. Not only diseases of Phanerogams, but those of Eumycetes as well are discussed; this is the case even if a fungus, attacked by a bacterium, is itself a pathogen of a higher plant. Bergey's Manual (1948) and Elliott's Manual of Bacterial Plant Pathogens (2nd ed., 1951) were used for bacterial nomenclature. This eliminates older confusion in the naming of causative agents and adds considerably to the international value of this volume.

It is difficult to do justice in less than several pages to the many topics covered in this volume. The documentation is unbiased and very complete, as can be seen, for instance, in the excellent discussion of crown gall. There are numerous illustrations throughout the text in the form of clear photographs and microphotographs. In a book which is mainly descriptive, good illustrations are a prime necessity, and the ones in this volume are excellent and therefore greatly increase the value of the book.

This imposing work will be indispensable to plant pathologists throughout the world, and will be consulted often and treasured because of its extensive literature citations.—Karl Maramorosch, The Rockefeller Institute for Medical Research.

A Flora of the Marshes of California. By Herbert L. Mason. 878 pages. Profusely illustrated. University of California Press. 1957. $10.00.

Sponsored jointly by the California Department of Fish and Game, the Federal Fish and Wildlife Service of the United States Department of the Interior, and the Department of Botany at the University of California, this work was designed as a practical manual for identification of marsh plants located along the major flyways of aquatic birds in California.

Rapidly increasing problems relating to changes in waterfowl habitats, through drainage of marshes and their conversion to farm lands, with consequent decrease of natural food plants and increase of waterfowl depredations on field crops, have rendered essential to the game manager a manual by which he can determine the plant sources of wildfowl food in his area, and thus plan the necessary steps toward correction.

The term "marshes" is here used broadly, the author stating that he has included all the species of the state known to him "to occur typically or commonly on wet lands or in water", with certain marginal plants of significance in waterfowl nutrition, added. Definite types of wet habitats—e.g. lakes, swamps, alkaline marshes, and bogs—are discussed and defined in the introduction; and habitat citations for each species described in the body of the Flora are specific. Geographic range for each is likewise given, not only within California but beyond its borders, adding value to the book for use in other areas of the West.

The diagnostic keys for families, genera, and species, are based upon readily ascertained characters, and most of the terms employed are illustrated by helpful line drawings. Descriptions are ample, and a partial synonymy is included. The illustrated glossary, also, is of much assistance.

An outstanding feature which immediately catches the eye is the artistry, as well as the technical accuracy, of 362 full-page plates of beautiful line drawings. Such a combination, with the accompanying excellence of text, is not always to be found in scientific publications.

In the preparation of a work such as this, which should be usable alike by the botanist and the layman, the author has the always recognized problem of the use of common names. He is to be commended here in avoiding the manufacture of such names where none exist; but he has perhaps omitted some which would have added to the consistency of his text. For example, it would appear as logical to associate Buttercup Family with *Ranunculaceae* and Buttercup with *Ranunculus* as Chickweed or Pink Family with *Caryophyllaceae*, or Smartweed with *Polygonum*.

Another minor suggestion is that certain terms which are used, without definition, in the introduction, be added to the glossary. *Lacustrine* and *riparian*, for instance, are absent; while *palustrine* and *paludose* are defined.

The above small items, however, are inconsequential in comparison with the excellence of the book as a whole. The author, his colleagues, and the artists, are alike to be congratulated on an outstanding contribution in the field of Botany.—HELEN M. GILKEY.

Biology and Control of the Smut Fungi. By George W. Fischer and Charles S. Holton. 622 pp. Illus. The Ronald Press Company. New York. 1957. $10.00.

BIOLOGY AND CONTROL OF THE SMUT FUNGI incorporates a few features of the two previous volumes on the Ustilaginales written by the senior author. However, many additional phases of work on this important fungal group are discussed in this monograph although a few subjects i.e. phylogeny and nomenclature are only briefly considered. This book, with or without the two earlier volumes, will prove very useful to the teacher and student of the smut fungi.

The binding appears to be the same green cloth used on the two previous smut volumes published by the Ronald Press Company. In this volume, however, the binding appears to be too weak for the greater number of heavy pages. The book is legibly printed

on heavy, gloss paper. The volume is well illustrated and many fine reproductions of old plates are presented. A bibliography of 46 pages testifies to the extensiveness of the literature coverage by the authors. Literature references in the text are made by author and date. In the bibliography the articles are listed alphabetically by author, and papers by the same author are listed chronologically under his name. A large, complete index and table of contents increase the usefulness of this volume which simplifies the finding of specific information in the text.

The authors present their aims in writing this book in its preface. If they had limited themselves to these goals, the book would have been useful. However, the authors go beyond their expressed intentions and present to the reader previously unpublished data, personal experiences, analyses and opinions of the information presented in the literature, and summaries at the ends of many of the sections within each chapter. These comments and thought-provoking ideas, clearly indicated in the text as authors' opinions, are liberally but unobtrusively presented throughout this volume resulting in the stimulation of original thinking on the part of the reader. Such a book as this is a privilege and a pleasure to read.

The chapters of the text are general biological classifications i.e. morphology, taxonomy, and symptomology, history and economic importance, nomenclature and phylogeny etc. Information about the order Ustilaginales is thoroughly presented over the entire range of biological subjects and includes discussions of the smut organisms, major and minor interactions of host and parasite, and methods of research and controls. One chapter discusses the effects of the smut fungi themselves on man and animals.

No attempt is made by the authors to sidestep controversial issues about which they feel qualified to comment. The reader will find major points of disagreement i.e. the taxonomic treatment by Fischer or, because of more intimate contact with a specific smut, minor points of disagreement i.e. symptoms caused by a specific smut. These controversial points do not reduce the value of the book. On the contrary, the authors have frequently pointed out such areas of disagreement as well as areas where information is partially or totally lacking.

This book presents a thorough, scholarly account of the total accumulated knowledge of the smut fungi. Since the literature for the smut fungi is widely distributed in many scientific journals in different languages, this monographic treatment of the smut fungi not only presents the world literature in a readily available form, but stimulates the reader to develop thoughts and ideas for future work. This volume should serve as a pattern for future monographic treatments of biological literature.—RICHARD L. KIESLING, Michigan State University.

NOTE

Dr. William Jacob Robbins, after twenty years as Director of The New York Botanical Garden, retired on 31 December 1957. During his administration many changes have taken place in the physical aspect of the Garden, the most conspicuous of which has been the construction of a new building for laboratory research. This is devoted chiefly to plant physiology and biochemistry; the development of these and allied disciplines reflects Dr. Robbins' own interests and activities.

Dr. William Campbell Steere has accepted appointment as Director in Dr. Robbins' place; he will take office on July 1. Dr. Steere is Professor of Biology and Dean of the Graduate Division of Stanford University. Previously he was Chairman of the Department of Botany of the University of Michigan. He is widely known for his researches in the taxonomy of mosses, and has traveled and collected in many parts of the Americas from the Andes to the Arctic. For the period from January to July Dr. David Daniels Keck, Head Curator of the Garden since 1951, will serve as Acting Director.

Dr. Clark Thomas Rogerson has been appointed Curator of the Cryptogamic Herbarium at the Garden. Before coming to New York on February 1, he was Assistant Professor of Botany at Kansas State College and Assistant Mycologist at the Kansas Agricultural Experiment Station. He takes the place left vacant by the removal of Dr. Donald Philip Rogers to Urbana, Illinois, where the latter is Professor of Botany in the University of Illinois. Dr. Rogers was recently Vice-President of the Club.—H.W.R.

BULLETIN OF THE TORREY BOTANICAL CLUB
Vol. 85, No. 3, pp. 220–230 MAY, 1958

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COMPILED BY

LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITOR OF BRITTONIA

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